Interactions of Histidine and Other Imidazole Derivatives with Transition Metal Ions in Chemical and Biological Systems

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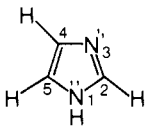
Contents

I. Introduction

The imidazole ring, as a histidine moiety, and the benzimidazole ring, particularly as its 5,6-dimethyl derivative, function as ligands toward transition metal ions in a variety of biologically important molecules including iron–heme systems, vitamin B_{12} and its derivatives, and several metalloproteins. The purpose of this review is to summarize information available on ligand–metal interactions in complexes containing imidazole and benzimidazole rings as ligands and, where possible, to discuss the relationship between the properties of these heterocyclic ligands and their function in biological systems. The discussion of the imidazole ring in biological systems is restricted to cases where there is considerable evidence that interaction with a transition metal ion is involved. Thus, this review does not discuss enzyme systems where imidazole rings are known to function as a nucleophile or in proton-transfer processes if there is no interaction with a transition metal ion. Although an exhaustive literature search for all imidazole-containing complexes was not attempted, an effort has been made to survey papers published up to mid-1973 which deal primarily with the structure and reactivity of transition metal complexes containing imidazole derivatives.

II. Properties of the Imidazole Ring

The structural features associated with the imidazole or 1,3-diazole ring must be discussed briefly. The parent molecule falls in the class of the aromatic heterocycles, and its unique structural features are conveniently discussed with reference to pyridine and pyrrole, to both of which imidazole is structurally related. Aromaticity in completely conjugated monocyclic systems requires a planar array of atoms with $4n + 2$ π electrons.[1] The possibility for aromaticity in imidazole can then be recognized if imidazole is considered to be constructed from a trigonal nitrogen with two electrons in the unhybridized p orbital (N-1, "pyrrole nitrogen"), a trigonal nitrogen with a lone pair in a hybrid orbital and a single electron in the p orbital (N-3, "pyridine nitrogen"), and three trigonal carbons each with one electron in a p orbital. An aromatic sextet is then available.



Imidazole is indeed generally regarded as being aromatic. The molecule is planar, as anticipated for an aromatic system.[2] By Dewar's definitions and procedures the resonance energy of imidazole is 15.4 kcal/mol.[3] The same method yields resonance energies of 20.0, 20.9, and 8.5 kcal/mol for benzene, pyridine, and pyrrole, respectively. Similarly, the resonance energies of benzimidazole (30.9 kcal/mol) and naphthalene (30.5 kcal/mol) are comparable. Empirical resonance energies for imidazole derived from thermochemical data range from 12 to 32 kcal/mol depending upon the assumptions taken.[4]

The simple description of the nature of the bonding in imidazole is qualitatively supported though quantitatively modified by all electron, *ab initio* (with a contracted Gaussian basis set) calculations performed on pyrrole,[5] pyridine,[6] and imidazole.[7] The net charges at the nitrogen atoms found in calculations for the three heterocyclic molecules are collected in Table I.

From a comparison of the values in Table I, the N-3 nitrogen of imidazole is aptly termed the pyridine nitrogen while the N-1 nitrogen is properly called the pyrrole nitrogen. The pyridine nitrogens display fractional negative σ and π electronic charges indicating that this nitrogen is a modest σ acceptor and a weak π acceptor. An important conclusion of the *ab initio* calculations, evident in Table I, is the two-way charge transfer at the pyrrole nitrogens. The pyrrole nitrogens donate substantial fractional electronic charge to the π system but withdraw an even greater amount of charge from the σ orbitals so that the total result is a gain of -0.44 electronic charge at the pyrrole N-1 nitrogen of imidazole and -0.41 of an electron at the nitrogen of pyrrole. When the N–H atoms of the pyrrole nitrogens are considered together, the total net σ charges are -0.47 and -0.41 of an electron in im-

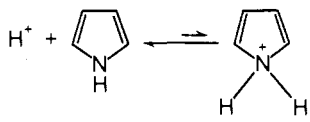
TABLE I. Calculated Net Charges at Nitrogen Atoms

	σ	π
Pyridine[a]	−0.22	−0.01
Imidazole[b]		
N-3	−0.16	−0.10
N-1	−0.84	+0.40
Pyrrole[c]	−0.75	+0.34

[a] Reference 6. [b] Reference 7. [c] Reference 5.

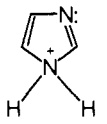
idazole and pyrrole, respectively. In both compounds the total net σ and π electronic charge of the N–H unit is −0.07. The calculations also indicate that the σ electrons of pyrrole and imidazole are strongly delocalized and polarized about the rings. Thus molecular orbital calculations that rely on an undistorted core of σ electrons when deducing π-electron properties of these compounds appear incomplete. The *ab initio* calculations on imidazole also indicate that all hydrogen atoms are σ donors as is C-2 to a lesser extent. Carbon atoms 4 and 5 of imidazole are σ acceptors. All the carbon atoms are weak π acceptors, but polarization of the σ electrons dominates the overall charge distribution.

A crucial structural feature with respect to the coordination site of imidazole is clarified when the aromatic nature of the molecule is recognized. There is only one pair of electrons properly described as an unshared pair, the pair on N-3. The π electrons of N-1 are part of the aromatic sextet. Bonding of a proton or metal ion at N-1 is expected to be very unfavorable since the aromaticity of the ring is thereby compromised. The basicity at this type



(1)

of site toward a proton cannot be measured in pyrrole because protonation at carbon intervenes with a pK_a of −3.8.[8] It has been estimated that the equilibrium constant for the reaction in eq 1 is 10^{-10} ($pK_a = -10$).[9] The pyrrole nitrogen of imidazole should be of comparable, or probably lower, basicity because of the additional electron-withdrawing effect of the pyridine nitrogen. The important conclusion is that the neutral imidazole molecule presents a single energetically favorable coordinating site for a proton, the unshared pair on N-3, and the same is most likely true for a metal ion. Structures **1** and **2** must be of very high energy. The transfer of the N-1 proton to



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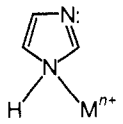
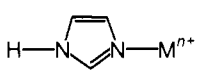


2

N-3 would be very favorable energetically, and species such as **1** or **2** would not be expected to be observable. The structures of the protonated and metal ion complexes of imidazole are the aromatic cations **3** and **4**,

3



4

Distinction between the N-1 and N-3 nitrogens is lost in the protonated imidazolium cation. Since this cation is symmetrical, H-4 and H-5 are equivalent, and a single peak appears in the proton magnetic resonance spectrum. A single averaged peak for the H-4 and H-5 protons also persists into basic solutions of neutral imidazole because proton-exchange reactions between N-1 and N-3 are rapid compared to the chemical shift difference between H-4 and H-5. In aqueous solutions tautomeric equilibration of the nitrogen bound hydrogen occurs without disruption of the aromatic electronic structure *via* proton-exchange reactions with solvent species. The predominant exchange reaction in acidic solutions is

$$ImH^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Im + H^+ \qquad (2)$$

and in basic solutions it is

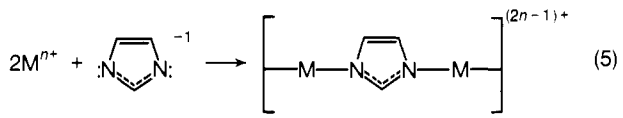
$$Im + H_2O \underset{k_4}{\overset{k_3}{\rightleftharpoons}} ImH^+ + OH^- \qquad (3)$$

Rate constants for the reverse reactions, k_2 and k_4, have been measured at 25° and the values furnished near-zero ionic strength.[10] The reverse reactions are favored thermodynamically, and the rate constants exhibit values typical of diffusion-controlled reactions;[11] $k_2 = 10^{10.2}$ sec^{-1} M^{-1} and $k_4 = 10^{10.4}$ sec^{-1} M^{-1}. Since under the same conditions, $10^{-7.0}$ $M = K_a = k_1/k_2$, calculation yields $k_1 = 10^{3.2}$ sec^{-1} and $k_3 = 10^{3.4}$ sec^{-1}. The near-equality of these two low rate constants is due to the nearness of the acidity constant K_a to 10^{-7} which is $K_w^{1/2}$. For this reason imidazole is an optimum catalyst and buffer.[11] The rate constant, k_3, for the protonation of neutral imidazole by water is the slow step in exchange of nitrogen bound hydrogens by solvent components in basic solutions of imidazole. The protonation reaction may be speeded up if general acid catalysts are present. An example of a general acid is the imidazolium cation, and for the reaction in eq 4 the forward and reverse rate constants should be about $10^{8.5}$ sec^{-1} M^{-1}.

$$Im + ImH^+ \rightleftharpoons ImH^+ + Im \qquad (4)$$

Because an aquated proton quickly penetrates hydrogen-bonded species, substitution of the bound water is not rate limiting in proton-transfer reactions such as the protonation of imidazole. In contrast, substitution of bound water in the divalent first-row transition metal ions is rate limiting with a rate constant characteristic of the metal ion and not of the monodentate ligand involved. Ligand dependency does appear with some chelatable ligands such as histidine and metal ions with fast coordinated water replacement rates such as Cu(II).[12] Substitution reactions at metal ions by imidazole and derivatives are reviewed more fully in sections III and IV.

In addition to protonation at N-3 with $pK_a = 7.1$ (25° and about 0.2 ionic strength) to give the imidazolium cation, neutral imidazole undergoes deprotonation at N-1 in strongly basic solutions with reported pK_a values from 14.2 to 14.6.[13–15] The resulting anionic imidazole, also aromatic, possesses two equivalent sites for coordination and is a potential bridging ligand. Reviews of solid com-



(5)

plexes of the imidazole anion appear in section III and metal ion induced deprotonation of the pyrrole hydrogen in section IX.

Although it is never a major component of the mixture in aqueous solution at equilibrium, the ylide formed by deprotonation of the imidazolium ion at C-2 can play a significant role in the chemistry of imidazole. The exchange of the proton at C-2 in imidazole in aqueous solu-

tion is quite rapid. The half-life for incorporation of deuterium from D_2O at 65° and pD 6.9 is 109 min.[16] pH-rate profiles for both imidazole and 1-methylimidazole[17] indicate that the ylide is the reactive species in the exchange process. Proton-exchange processes at C-2 of 1,3-dialkylimidazolium ions have also been studied and found to occur *via* ylide intermediates.[18,19] The most stable ylide is formed by deprotonation at C-2, which is bonded to two positively charged nitrogens. The 4(5) position adjacent to a single nitrogen is much less acidic.[16]

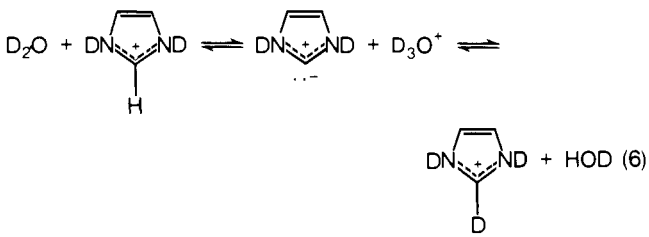


Figure 1 summarizes the protonation equilibria for imidazole and gives similar data for histidine and benzimidazole. The abbreviations given in the figure for the various species are those which will be used subsequently in the text.

In qualitative discussion of the imidazole ring as a ligand, it will often be convenient to compare it with ammonia and pyridine. There are considerable parallel data on both of these ligands. Imidazole possesses two properties, basicity and π-electron acceptor capability, that are intermediate between those of saturated amines such as NH_3 and aromatic amines such as pyridine. In comparing an imidazole-containing system and the corresponding one with saturated or pyridine amines, recourse is often made to relative basicities and π-electron acceptor capabilities. The order of increasing basicity, pyr < Im < NH_3, is also the order of decreasing π-electron acceptor capability, which is nil for NH_3. The reverse ordering of the two properties through the three kinds of ligands allows easy explanations for observations where the above order or its reverse occurs. An order pyr < Im < NH_3 indicates that basicity and/or inability to accept π electrons are the dominant factors. The reverse order NH_3 < Im < pyr suggests that π-electron acceptor capability and/or weak basicity are more important. Frequently observations lead to an order where imidazole heads the list, NH_3, pyr < Im. In these cases some combination of the two properties evidently contributes to the observed order. For example, an effect which depends upon an appropriate combination of basicity and π-acceptor capability may yield the order NH_3 < pyr < Im because NH_3 exhibits negligible π acceptor capability and Im possesses the best combination of the two properties. Another weighting of the two properties may give the usual stability constant order, pyr < NH_3 < Im, discussed in the next section. The principles summarized in this paragraph are frequently applied, and examples occur throughout this article.

III. Imidazole and Benzimidazole Complexes

As noted in Figure 1, imidazole and also benzimidazole are amphoteric, being moderately strong organic bases capable of accepting protons at N-3 as well as very weak acids capable of loss of a proton from N-1. As will be evident from the discussion to follow, in solutions near neutrality the unprotonated imidazole molecule usually functions as a ligand *via* the unshared pair of electrons on N-3. Although structures involving bonding with the "pyrrole" nitrogen of imidazole have been proposed[21] or considered as reaction intermediates[22] and there seems to be fairly widespread acceptance of this possibility for two bonding modes,[23,24] there is no authenticated case of such bonding and it is *a priori* quite unlikely, as pointed out in section II, because an "unshared pair" does not exist at the pyrrole nitrogen as the electrons are delocalized throughout the π system.

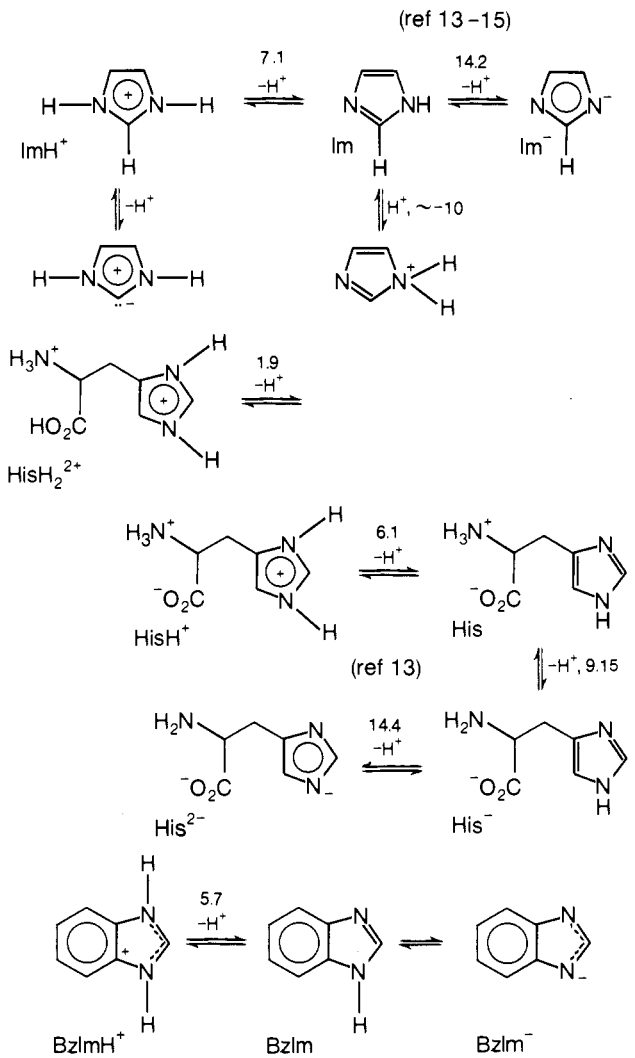


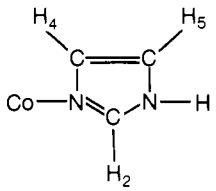
Figure 1. Equilibrium constants and abbreviations for imidazole, histidine, and benzimidazole. Data are from ref 20 except as noted. Equilibrium constants are expressed as pK_a values above the arrows.

Freeman and Szymanski have commented on the absence of any four-coordinate nitrogen atoms in crystalline substances containing the imidazole ring as a ligand and pointed out that the prohibition against this structural type must come from a requirement to retain the aromaticity of the imidazole ring.[25]

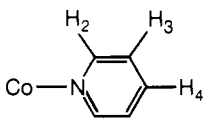
Surveys[26,27] of a number of the most commonly studied transition metal ions have established that Mn(II), Fe(II), Co(II), Ni(II), and Cd(II) form crystalline products of composition $M(Im)_6X_2$ where X includes common anions such as Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, or BF_4^-. Cu(II) usually gives $Cu(Im)_4X_2$. Compounds with the stoichiometry $Cu(Im)_6I_2$ and $Cu(Im)_6(NO_3)_2$ have been prepared,[27] but these are formulated as $Cu(Im)_4X_2 \cdot 2Im$ with two imidazoles occupying uncoordinated lattice positions. In the case of Zn(II) both $Zn(Im)_4X_2$[26] and $Zn(Im)_6X_2$[27] species have been described. Crystalline $Zn(Im)_6Cl_2 \cdot 4H_2O$ obtained from $ZnCl_2$ and excess imidazole has been shown to contain $Zn(Im)_6^{2+}$ cations by a crystal structure determination.[28] Other ions of formula

$M(Im)_6^{2+}$ have been assigned octahedral geometry on the basis of their electronic spectra,[26,29,30] and these assignments have been confirmed by crystal structures in several cases. Spectra of these octahedral ions place imidazole above oxygen donors and just below ammonia and pyridine in the spectrochemical series and well below the chelating amines such as ethylenediamine and 2,2′-bipyridine in ligand field strength. Magnetic susceptibility measurements are also consistent with expectation for an octahedral arrangement of the imidazoles in the hexakis complexes. A detailed analysis of the magnetic properties of $Co(Im)_6(NO_3)_2$ has been presented.[31] When heated *in vacuo* above 100°, $Co(Im)_6(ClO_4)_2$ loses imidazole, giving $Co(Im)_4(ClO_4)_2$, in which the coordination at cobalt is tetrahedral.[29] The crystalline copper complexes of composition $Cu(Im)_4X_2$ have been formulated as distorted octahedra with the anions occupying the apical positions.

An nmr study comparing aqueous acetone solutions of Co(II) with pyridine and imidazole as ligands indicates a preference for binding of four and six molecules, respectively.[32] The technique used involved observation of the pmr signals of bound ligands at temperatures at which the ligand exchange rate is slow on the nmr time scale. Because of the paramagnetic Co(II) ion, large isotropic shifts are observed for the bound ligands. Quantitative estimation of the number of bound ligands was made from integration data. A surprising feature of the signals of the complexed imidazole molecules is that apparent equivalence of the H-4 and H-5 hydrogens of the imidazole molecule was observed. Since binding to cobalt necessarily distinguishes between the two nitrogens, the H-4



and H-5 protons would not be expected to be equivalent. This is particularly true because of the large isotropic shifts caused by the paramagnetic cobalt ion. For example, there is a difference of 26.5 ppm between the H-2 and "H-4, H-5" signals of imidazole complexed to Co(II). Furthermore, complexation of pyridine to Co(II) causes a strong differentiation of the isotropic shift for the 2, 3, and 4 protons.



Δ_2	Δ_3	Δ_4 (ppm)
−107.5	−31.0	+10.0

If the isotropic shifts are mainly scalar (contact) in origin, the broadening of a resonance line should be proportional to its shift. This result was observed for pyridine but not imidazole ligands, a difference suggesting that the resonances in the latter case may not be properly identified and located. The 2:1 histidine complex of Co(II) shows the H-4 resonance at about +50 ppm,[33] and a corresponding upfield resonance might be expected for the H-5 of imidazole that is complexed to Co(II) and undergoing slow exchange. Nmr studies of DMSO solutions of Co(II) and imidazole show formation of complexes in which the expected large contact shift of the imidazole protons is observed.[34] This study was done under conditions of fast exchange of the ligand from complexed to uncomplexed environment, and no chemical shift between the C-4 and C-5 protons is to be expected.

The tendency of imidazole to form hexakis complexes has been commented on by several groups and stands in interesting contrast to the well-studied case of pyridine where the preference is for tetrakis substitution.[27,35,36] Two points have been brought forward as explanations for this difference. Since the π-acceptor ability of imidazole is less than that of pyridine, the charge buildup on the metal ion with successive addition of imidazoles may disfavor entry of negatively charged ions such as halide into the first coordination sphere. All coordination sites are, therefore, filled by neutral imidazole molecules. The electron density increase at the metal ion would be less in the case of the pyridine system because of the more effective π-acceptor properties of pyridine.[35]

Ligand size has also been proposed as the dominating factor in determining whether hexakis complexes will form. We may assess the importance of ligand size by noting that the bond angle at nitrogen is 116° 50′ in pyridine[37] while the average angle at N-3 is 108° in a collection of imidazole complexes.[38] Including the relevant bond lengths we calculate the distance between the pair of ortho carbons in pyridine to be 2.28 Å; this cross ring distance widens to 2.39 Å at the meta carbons. For complexed imidazole the distance between C-2 and C-4 is about 2.18 Å while this dimension narrows to 1.37 Å between C-5 and N-1. However, it is the cross ring distance between the close-in hydrogens ortho to the complexed nitrogen that should be most important in producing steric effects. If we assume that the C–H bond lengths are equal in the two ligands, the distance between ortho hydrogens is about 0.10 Å greater in pyridine than imidazole. Though this difference might be critical in a few cases, just a slight rotation of the ligand ring plane easily reduces any hindrance due to interaction of ortho hydrogens. It appears that steric hindrance is insufficient to account for most instances of six-coordination with imidazole and four-coordination with pyridine.

That imidazole shows a greater tendency to form hexakis complexes than pyridine is apparent from the stability constants listed in Table II. We consider Ni(II) as the most favorable case for hexakis complexes and extrapolate the trends in Table II to obtain log $K_5 \simeq 1.0$ for imidazole and $\simeq -1.0$ for pyridine. The smallness of the equilibrium constant for association of the fifth pyridine ligand, $K_5 \simeq 0.1$, may be appreciated by applying, for illustrative purposes only, the constant to neat pyridine, which is about 12 *M* in ligand, with the result that the molar ratio of 5:1 to 4:1 complexes is only 1.2. By contrast the equilibrium constant $K_5 \simeq 10$ for imidazole indicates that even in an aqueous solution which is 1.0 *M* in unbound imidazole the ratio of 5:1 to 4:1 complexes is 10.

Two factors contribute to the 160 times greater equilibrium constant for imidazole over pyridine in formation of 5:1 complexes with Ni(II). First, imidazole is 80 times more basic than pyridine so that first stability constants are substantially greater for imidazole. Second the interval between successive stability constants of Ni(II) is greater for pyridine, resulting in an additional lowering for each additional ligand added.

Representative logarithms of stability constants for complexation of metal ions with ammonia, imidazoles, pyridine, and histidine are listed in Table II. In order to maintain consistent conditions when comparing stabilities of a single ligand with a variety of metal ions, the classic work of Bjerrum was used for ammonia and most pyridine constants. The values for ammonia are at greater

TABLE II. Logarithms of Stability Constants

		H^+	Co(II)	Ni(II)	Cu(II)	Zn(II)	Cd(II)	Hg(II)	Cu(I)	Ag(I)
NH_3[a]	K_1	9.33	2.11	2.80	4.15	2.37	2.65	8.8	5.9	3.2
	K_2		1.63	2.24	3.50	2.44	2.10	8.7	4.9	3.8
	K_3		1.05	1.73	2.89	2.50	1.44	1.0		
	K_4		0.76	1.19	2.13	2.15	0.93			
4-Methylimidazole[b]	K_1	7.69			4.13	2.44				
	K_2				3.49	2.53				
	K_3				2.87	2.64				
	K_4				2.0	2.4				
Imidazole[c]	K_1	7.11	2.45	3.0	4.20	2.52	2.80		5.8	3.1
	K_2		1.9	2.5	3.42	2.32	2.10	β_2 16.7	5.2	3.8
	K_3		1.4	2.0	2.88	2.32	1.55			
	K_4			1.5	2.1	2.0	1.1			
Pyridine[d]	K_1	5.21	1.15	1.78	2.41	0.88	1.30	5.1	3.2	2.0
	K_2		0.55	1.22	1.88	0.47	0.84	4.9	3.4	2.2
	K_3		−0.3	0.3	1.14	0.15	0.36	0.3		
	K_4			−0.3	0.60	−0.2	−0.2			
Histidine[e,e]	K_1	9.15	6.9	8.7	10.1	6.6	5.4			
	K_2	6.10	5.5	6.9	8.0	5.5	4.3	β_2 21		
Histidine methyl ester[f]	K_1	7.30		6.19	8.48	4.46	3.98			
	K_2	5.35		4.91	5.90	4.20	2.81			
	K_3			2.90	1.6	0.0	~1			
Histamine[g]	K_1	9.88	5.27	6.88	9.55	5.62				
	K_2	6.13	3.68	5.03	6.48					
	K_3		2.0	3.1						

[a] All values in 2 *M* NH_4NO_3 and 30°, except Hg(II) and Cu(I) which are near 20°; J. Bjerrum, *Chem. Rev.*, **46**, 381 (1950). [b] At 25° and 0.16 ionic strength: Y. Nozaki, F. R. N. Gurd, R. F. Chen, and J. T. Edsall, *J. Amer. Chem. Soc.*, **79**, 2123 (1957). [c] "Consensus" values near 0.2 ionic strength and 25° (where possible) from "Stability Constants," Special Publications 17 and 25, The Chemical Society, London, 1964 and 1971. [d] Except for Cu(I) all values at 25° and 0.5 ionic strength: J. Bjerrum, *Acta Chem. Scand.*, **27**, 970 (1973); **26**, 2734 (1972); **18**, 843 (1964); Cu(I) at 20° and 0.15 ionic strength; ref 101. [e] At 25° and 0.10 ionic strength; ref 131 and 157. [f] At 25° and 0.16 ionic strength; ref 116. [g] At 25° and 0.14 ionic strength: B. L. Mickel and A. C. Andrews, *J. Amer. Chem. Soc.*, **77**, 5291 (1955); ref 118.

ionic strength and temperature than the constants for the other ligands of Table II. The ionic strength dependence of the constants is slight, and the 5° greater temperature is a constant difference in comparing ammonia with other ligands. For the ligands of Table II the usual stability order[39] Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) is found. Changes in vibrational frequencies found in infrared spectra have been interpreted to give the same stability order.[26,40]

Comparisons of stability constants among ligands require some recognition of their differences in basicity. Omission of this important factor has often resulted in faulty conclusions. The precise dependence of stability constants on ligand basicity varies among families of ligands and also from ligand to ligand. Table II shows that stability constants for divalent transition metal ions are slightly greater for Im than NH_3 despite the 170 times greater basicity of the latter. (Introduction of statistical factors does not alter the qualitative conclusions.) If σ-bonding ability were the sole factor determining stability constants, the values for NH_3 would be appreciably greater than those for Im. The relatively strong binding of the weaker base Im to metal ions may be at least partly ascribed to its greater π-acceptor properties, which permit it to accept electronic charge from d orbitals on the metal ion (t_2 orbitals for octahedral complexes). It is the occurrence of π-type interactions between ligand and metal ion that is frequently responsible for the lack of an exact correspondence between stability constants and ligand basicity.

The importance of π-acceptor qualities in strengthening the bonding of imidazole to metal ions may be further supported by noting that though 4-methylimidazole is nearly a four times stronger base than imidazole, it binds to Cu(II) and Zn(II) less strongly. This inversion of order in pK_a and log K_1 for the two imidazole ligands may be accounted for by the hyperconjugative effect of the methyl group which reduces the π-acceptor capability of the imidazole ring. Significant π-acceptor properties remain in 4-methylimidazole as the stability constants for Cu(II) and Zn(II) are nearly identical with those for NH_3, while the latter ligand binds the proton over 40 times more strongly. 1-Methylimidazole and imidazole exhibit nearly identical pK_a and stability constant values for Cu(II), Cd(II), and Ag(I), the only three metal ions for which values have apparently been reported.[41,42]

As pointed out in section II, the π-acceptor capability of pyridine is greater than that of imidazole, and for most of the metal ions of Table II the stability constants of pyridine are greater than its basicity difference alone with imidazole would suggest. Moreover, the nearly constant difference in log K_1 between Im and NH_3 does not carry over to pyridine, where the differences with both of the other two ligands varies with the metal ion. The first four ligands of Table II are arranged in order of decreasing basicity, which is also the order of increasing π-acceptor capability. The latter feature apparently completely dominates the former with Ru(II), a low-spin d^6 ion. Estimates of the stability constant for formation of complexes of Im, NH_3, and pyridine with pentaammineruthenium(II) have been made from kinetic data.[43,44] The stability order pyridine > Im > NH_3 stands in striking contrast to the order Im > NH_3 > pyridine found with the first transition metal series. The promotion of pyridine can be attributed to the exceptional π-donor ability of Ru(II).[45,46]

There are no extensive data on formation constants in nonaqueous media. Wang and Li have estimated β_4 for formation of $Zn(Im)_4^{2+}$ in DMSO as 1.4×10^6, from the chemical shift of the imidazole 2H as a function of Zn(II) concentration.[47] They note that this value is substantially lower than the value found in aqueous systems. Enthalpy data available for imidazole complexes have been tabulated by Ashcroft and Mortimer.[48] Data are available for Ni(II), Cu(II), Zn(II), and Cd(II).

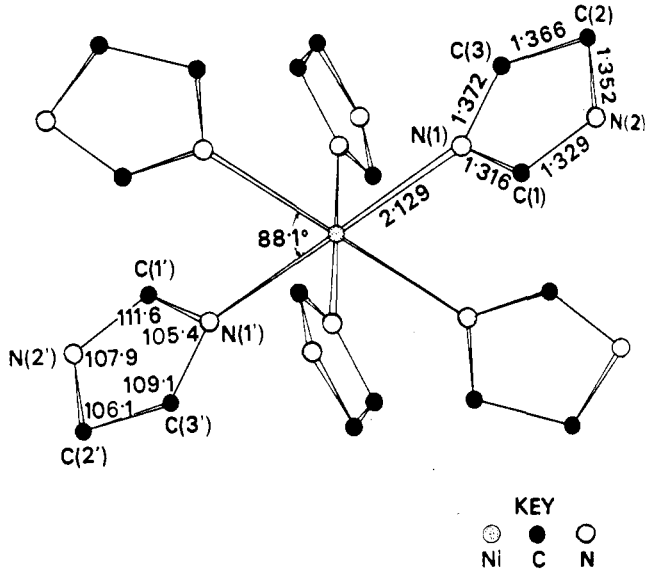


Figure 2. The $Ni(Im)_6^{2+}$ cation in $Ni(Im)_6(NO_3)_2$. Reproduced from ref 49.

A number of imidazole-transition metal complexes have been subjected to crystallographic structure determination. In the case of hexakis-substituted systems, nitrate salts of Ni(II),[49] Cd(II),[23] and Co(II),[50] a hydrated dichloride of Zn(II),[28] a hydrated carbonate of Co(II),[51] and a "hydroxide nitrate tetrahydrate" of Cd(II)[23,52] have been studied. The structures of the three nitrate salts are very similar. The cation has threefold symmetry with a slight compression along one of the threefold axes of a regular octahedron. Imidazole groups in a trans arrangement are coplanar, and the metal ion also lies in this plane. In each structure this plane makes an angle of 24° with a plane defined by the metal atom, the trans imidazole nitrogens, and a second pair of imidazole nitrogens. The Ni(II) cation is depicted in Figure 2. The symmetry of the cation in the second $Cd(Im)_6^{2+}$ salt is similar but, in response to changes in anion packing, the relative orientation of the imidazole rings is changed, and the angle of intersection of a plane defined by a pair of trans imidazoles with a coordination square containing those imidazole nitrogens is 78°.[23] The octahedral coordination at Zn(II) in $Zn(Im)_6Cl_2 \cdot 4H_2O$ is less symmetric.[28] The Zn-N bond lengths range from 2.15 to 2.26 Å, and each imidazole ring has a slightly different orientation in this cation. Very roughly speaking (within 15°) each trans pair of imidazoles defines a plane which is perpendicular to one of the remaining axes of the octahedron and coplanar with the other. The structure of the $Co(Im)_6^{2+}$ unit in $Co(Im)_6CO_3$ is also less symmetric.[51] The imidazoles constitute three identical trans-oriented pairs, but the M-Im and ligand bond lengths are different between the two members of the pair.

Distorted octadedral coordination is present in $Cu(Im)_4SO_4$.[53] The four N-3 nitrogens of the imidazoles are coplanar with the Cu but slightly distorted from a square. Oxygens from sulfate complete the octahedral coordination. The imidazole rings are rotated relative to the coordination square. One pair makes an angle of 80°, the other 29°. A crystal structure determination $Cu(Im)_4I_2$ has been reported in preliminary form.[54] The coordinating imidazole nitrogens occupy essentially square-planar positions, the rings being oriented approximately perpendicularly to the plane of the coordinating nitrogen atoms. The iodides occupy relatively distant positions on a distorted octahedra. The stabilization of Cu(II) by imidazole in the presence of I^- is anticipated from consideration of redox potentials and the stability constants presented in Table II (section VIII).

A crystalline substance of composition $Cu(Im)_4(CH_3OCH_2CO_2^-)_2$ has also been examined by X-ray diffraction.[55] The imidazoles occupy four coordination sites in a plane, and the methoxyacetate ligands are bound at the axial sites with a Cu-O distance of 2.82 Å. The imidazoles are not all identical but represent two trans-oriented pairs. One pair involves typical metal ion-imidazole interaction *via* the pyridine nitrogen atom. The other pair of imidazoles appear to be the deprotonated Im^- species, and the Cu-N distance is quite short, being only 1.91 Å. The two protons required by the observed composition are considered to be associated with a group of three atoms, one nitrogen and a carboxylate oxygen and a methoxy oxygen, rather than with a single atom. The imidazole bond distances in this complex are significantly different from those found in most other studies of coordinated imidazoles. Though bonding of the pairs of trans imidazole groups to the Cu(II) differs, both pairs contain bond lengths of 1.46 Å within the imidazole ring which are abnormally long compared to most of those of other structures containing complexed imidazole moieties (Table IV below). This long bond length approaches that expected for a C-N single bond. It is not clear if this long bond represents a real alteration in the structure of the imidazole ring or simply is the result of unusual packing forces in this crystal.

A crystalline material of composition $Cu(Im)_2Cl_2$ is obtained from solutions at pH 5 when the Cu:Im ratio is about 1:2.[56] The copper is five-coordinate with two Im and two chloride ions at somewhat closer distances than a chloride which bridges to a second Cu. The Cu is significantly (0.18 Å) out of the plane of the four nearest neighbor ligands. The geometry of the imidazole ring is normal. With excess imidazole at pH 7.5-8, a salt $Cu(Im)_2(Im^-)(Cl)$ crystallizes, and it has also been examined by X-ray methods.[57] It contains polynuclear chains formed by bridging with imidazolate anions. Each Cu(II) ion is also coordinated by two neutral imidazoles and a chloride ion. The Cu-Im^- bonds (1.96-1.98 Å) are significantly shorter than the Cu-Im distance (2.06 Å), but the imidazole and imidazolate ring dimensions are in the usual range.

The structure of a perchlorate salt precipitated from alkaline solution of Cu(II) containing Im at greater than eight times the Cu(II) concentration has also been solved.[58] The composition of this substance is $Cu_3(Im^-)_2(Im)_8(ClO_4)_4$. Two types of Cu are present. One is coordinated by two imidazoles and two bridging imidazolate anions. The other type is coordinated by three imidazoles and one imidazolate bridge. The former type has essentially square-planar coordination, but the latter is appreciably distorted. The bond lengths in the imidazole rings vary from 1.30 to 1.40 Å and show no significant deviation from values found in most other imidazole complexes. The imidazolate bridging rings have similar dimensions.

A salt of composition $Co(Im)_2CO_3 \cdot H_2O$ has also been examined by X-ray methods.[59] Each Co is coordinated by two water molecules, a chelated carbonate, and a trans-oriented pair of imidazoles. The planes of the two imidazole rings are nearly perpendicular to one another and in an orientation such as to maximize d-pπ-overlap. However, a number of hydrogen bonding interactions are present in the crystal, and it is not clear that the orientation of the rings is a result of the metal-ligand π-bonding.

Cobalt is very nearly tetrahedrally coordinated in $Co(Im)_2Cl_2$.[60] The two Co-N bond length are just under

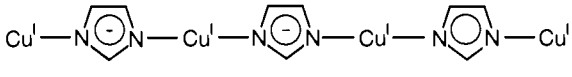
2.00 Å, somewhat less than in octahedral complexes. The only unusual ring dimension is a rather short C–C bond length in one ring. Preliminary results on $Co(Im)_2(CH_3CO_2)_2$ which establish a tetrahedral geometry at cobalt have been reported.[61]

Crystal data have also been obtained for $[Ag(Im)_2]NO_3$.[62,63] The geometry at silver is slightly distorted from diagonal with the N–Ag–N angle being 172°. The silver ion is significantly out of the planes defined by each of the imidazole rings. The bond lengths in the imidazole ring are in the normal range.

At lower imidazole metal ion ratios, products with fewer ligand molecules have been isolated.[27,29,35,64,65] Table III summarizes many of the known examples and indicates structural assignments. X-Ray data[66] are available for $Zn(Im)_2Cl_2$ and confirm a tetrahedral coordination about the zinc ion. The other structural assignments have been made on the basis of electronic or vibrational spectral data.

1-Methylimidazole and 1-butylimidazole both form a series of complexes with the first-row dipositive transition metal ions in which all but Cu(II) give the hexacoordinate derivative. Zinc gives both a tetracoordinate and hexacoordinate derivative.[67,68] Both tetrakis and pentakis 1-methylimidazole complexes of Rh(III) have been described.[69] A crystal structure determination has been reported on the dichloride salt of *trans*-diamminebis(1-methylimidazole)platinum(II).[70] A number of analogs in which 2-methylimidazole is the ligand have also been studied. These compounds show structural properties parallel to those observed with imidazole,[71-73] although the 2-methyl substituent introduces sufficient steric bulk that tetrakis substitution is found for the common divalent cations, except for Cd(II). The crystal structure of one of two isomeric compounds $Co(2\text{-}MeIm)_4(NO_3)_2$ has been determined.[74] A very distorted octrahedral coordination about the metal ion was found with a chelating nitrate occupying the fifth and sixth sites.

In sufficiently basic media the conjugate base of imidazole, Im^-, is formed and may function as a ligand. The tendency then is for formation of compounds of stoichiometry $M(Im^-)_2$ with dipositive metal ions. These materials have usually been found to be insoluble and generally are considered to be polymeric in nature. Examples reported include the Fe(II),[75] Cu(II),[42,76] Zn(II),[42,76] Co(II),[35,76] and Ni(II)[35,42] systems. The coordination around Zn(II) in $Zn(Im^-)_2$ and Co(II) in $Co(Im^-)_2$ is tetrahedral.[35] A distorted square-planar arrangement has been observed[77] for $Cu(Im^-)_2$ and postulated[35] for $Ni(Im^-)_2$. Three different forms of $Cu(Im^-)_2$ have been described.[78] These differ in color and also in magnetic behavior, but their detailed structures are unknown. The imidazolate salt of Cu(I) has also been prepared.[79] A polymeric bridge structure has been proposed. The com-



plexes of Im^- are usually prepared from the metal ion and imidazole in basic solution,[76] but $Zn(Im^-)_2$ has also been formed by reaction of dimethylzinc and imidazole (eq 7).[80] A salt which apparently contains the ion

$$(CH_3)_2Zn + ImH \longrightarrow Zn(Im^-)_2 + 2CH_4 \qquad (7)$$

$[Cr(Im^-)_6]^{3-}$ involving coordination of Cr(III) by six imidazole anions has been prepared by reaction of $CrCl_3 \cdot 3THF$ with the lithium salt of imidazole.[81]

$$CrCl_3 \cdot 3THF + 6LiIm \longrightarrow Li_3Cr(Im^-)_6 \cdot THF \qquad (8)$$

TABLE III. $M(Im)_xY_2$ Complexes

$M(Im)_x^{2+}$	Anion Y	Structural assignment	Ref
Mn(Im)	Cl, Br	Polymeric octahedra	27, 64
$Mn(Im)_2$	Cl, Br, I, NCS	Polymeric octahedra	27
$Mn(Im)_4$	Cl, Br, I, ClO_4, NCS	Distorted octahedra	27, 64
Co(Im)	Cl	Polymeric octahedra	65
Co(Im)	Br, I	Distorted tetrahedra	65
$Co(Im)_2$	Cl, Br, I	Tetrahedral	27, 35, 60, 65
$Co(Im)_2$	NO_2	Octahedral (chelating NO_2^-)?	27, 29
$Co(Im)_2$	NCS	Multiple forms	29
$Co(Im)_2$	NCSe	Polymeric octahedra	29
$Co(Im)_4$	ClO_4	Tetrahedral	29
$Co(Im)_4$	NCO, NCS, NCSe	Octahedral	29
Ni(Im)	Cl, Br	Polymeric octahedra	27
Ni(Im)	I	Distorted tetrahedra	65
$Ni(Im)_2$	Cl, Br	Polymeric octahedra	65
$Ni(Im)_2$	I	Distorted tetrahedra	65
$Ni(Im)_4$	Cl, Br, I	Octahedral or square	27, 65
$Zn(Im)_2$	Cl, Br, I	Tetrahedral	27, 66
$Zn(Im)_2$	NO_3	?	27
$Zn(Im)_4$	NO_3	Distorted octahedra	27

Other than the trinuclear $Cu_3(Im)_8(Im^-)_2(ClO_4)_4$ salt described earlier, no binuclear or other small polynuclear ions containing Im^- as the bridging ligand appear to have been characterized. The possibility of the occurrence of such bridging has been considered for two Cu(II) compounds, but the weight of evidence in each case led to rejection of such structures in favor of other formulations.[82,83] Mononuclear complexes containing a coordinated imidazole anion that have been characterized in solution are reviewed in section IX.

Some generalizations about the details of imidazole bonding can be made on the basis of the X-ray structures which have been reported. Some of these data are summarized in Table IV. Freeman and Szymanski[25] noted in 1967 that all nitrogen bound complexes of neutral imidazole derivatives were *via* the pyridine-type nitrogen. This generalization remains true. In complexes involving neutral monodentate imidazole ligands, the metal ion is usually nearly coplanar with the imidazole ring. A large deviation is found in the $Co(2\text{-}MeIm)_4(NO_3)_2 \cdot 0.5EtOH$ structure where the cobalt is nearly 0.4 Å out of coplanarity with one of the 2-methylimidazole rings. Smaller deviations are found in $Ag(Im)_2NO_3$ and $Cu(Im)_2Cl_2$. Other substantial deviations from coplanarity occur in $Cu_3(Im)_8(Im^-)_2(ClO_4)_4$, $Cu(Im^-)_2$, and $Zn(Im^-)_2$, where the ligand is the imidazole anion. In several histidine and peptide complexes, where chelation is involved, significant departure of the metal ion from coplanarity with complexed imidazole rings is again noted. The orientation of the imidazole ring relative to the coordination square is variable. In $Cu(Im)_4I_2$, the imidazole rings are apparently all approximately perpendicular to the plane defined by the coordinated nitrogen atoms. However, in $Cu(Im)_4SO_4$ and $Cu(Im)_4(CH_3OCH_2CO_2^-)_2$ the rings are inclined to the coordination square at angles ranging from 29 to 80°. In $Cu_3(Im)_8(Im^-)_2(ClO_4)_4$ coordination at one type of Cu is similar to that in $Cu(Im)_4I_2$, but at the other type of site the trans pairs of rings are titled with respect to one another and with respect to the coordination square.

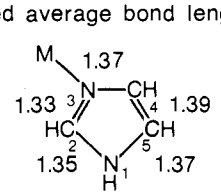
In $Ni(Im)_6(NO_3)_2$, $Cd(Im)_6(NO_3)_2$, and $Co(Im)_6(NO_3)_2$ the trans pairs of imidazoles all make angles of 24 and 66°, respectively, with the two coordination squares of

TABLE IV. Structural Data on Imidazole Complexes

Structure	Bond length, Å						∠Im plane to coordination squares[a]	Ref
	M–N$_3$	N1–C2	C2–N3	N3–C4	C4–C5	C5–N1		
Ni(Im)$_6$(NO$_3$)$_2$	2.13	1.33	1.31	1.37	1.37	1.35	24, 66	49
Cd(Im)$_6$(NO$_3$)$_2$	2.36	1.32	1.32	1.37	1.34	1.36	24, 66	23
Cd(Im)$_6$(OH)(NO$_3$)·4H$_2$O[b]	2.36	1.33	1.31	1.37	1.33	1.36	78, 12	23
Co(Im)$_6$(NO$_3$)$_2$	2.16	1.33	1.32	1.38	1.35	1.36	24, 66	50
Zn(Im)$_6$Cl$_2$·4H$_2$O[c]	2.15–2.26	1.28–1.53	1.32–1.38	1.31–1.53	1.33–1.42	1.23–1.51	75–90, 0–15	28
Co(Im)$_6$CO$_3$·5H$_2$O	2.16	1.28	1.29	1.34	1.48	1.34		51
	2.18	1.41	1.40	1.42	1.31	1.31		
Co(Im)$_2$(H$_2$O)$_2$CO$_3$	2.11	1.34	1.32	1.38	1.33	1.36	1.5, 88.5	59
Cu(Im)$_4$(CH$_3$OCH$_2$CO$_2^-$)$_2$[d]	1.91	1.30	1.39	1.38	1.36	1.46	64	55
	2.045	1.38	1.30	1.46	1.37	1.40	64	
Cu(Im)$_4$SO$_4$	2.00	1.34	1.33	1.37	1.37	1.36	29	53
	2.02	1.34	1.305	1.38	1.35	1.365	80	
Cu(Im)$_4$I$_2$	1.98–2.04						~90	54
Cu(Im)$_2$(Im$^-$)Cl	2.06	1.375	1.325	1.40	1.385	1.36		57
	1.96, 1.99[e]	1.33	1.33	1.36	1.365	1.40		
Cu$_3$(Im)$_8$(Im$^-$)$_2$(ClO$_4$)$_2$	1.99	1.39	1.33	1.36	1.39	1.36		58
	2.01							
	1.975[f]							
Cu(Im)$_2$Cl$_2$	1.99	1.36	1.39	1.38	1.34	1.38		56
	1.99	1.36	1.34	1.37	1.32	1.34		
Ag(Im)$_2$NO$_3$	2.12	1.36	1.32	1.37	1.335	1.39		62, 63
	2.13	1.31	1.32	1.36	1.385	1.42		
Zn(Im)$_2$Cl$_2$	1.995	1.35	1.32	1.37	1.37	1.39		66
	2.02	1.34	1.30	1.37	1.37	1.35		
Co(Im)$_2$Cl$_2$	1.99	1.36	1.31	1.42	1.39	1.41		60
	2.00	1.39	1.34	1.40	1.31	1.37		
Pt(NH$_3$)$_2$(1-MeIm)$_2$	2.01	1.27	1.36	1.38	1.30	1.41	49°	70
Co(2-MeIm)(NO$_3^-$)$_2$· 0.5EtOH[g]	2.25	1.34	1.32	1.36	1.35	1.33		74
	2.21							
	1.96							
	2.105							
Cu(Im$^-$)$_2$	1.99, 1.96	1.33	1.33	1.37	1.39	1.37		77
	2.00, 1.97	1.33	1.33	1.375	1.36	1.375		
Zn(Im$^-$)$_2$	2.01, 1.98	1.33	1.33	1.35	1.40	1.35		*h*
	1.98, 2.00	1.36	1.36	1.36	1.41	1.36		
	1.97, 1.99	1.35	1.35	1.37	1.41	1.37		
	2.02, 1.96	1.34	1.34	1.33	1.49	1.33		

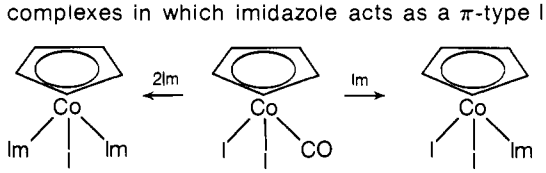
[a] When not given in the original paper these values were calculated by Mr. Bruce Blaylock. [b] The identification of the anion and water of hydration have been questioned; see ref 52. [c] Bond lengths for the nonequivalent pairs vary substantially from ring to ring. [d] Values given are for each of the two nonequivalent pairs of imidazoles. [e] Bridging imidazolate; the designations N-1 and N-3 are arbitrary. [f] Cu–Im$^-$ bond length. [g] Mean values of the imidazole bond lengths are given. [h] From the unpublished results of C. I. Bränden and C. Sandmark quoted in H. C. Freeman, *Advan. Protein Chem.*, **22**, 257 (1967).

which they are part. In Cd(Im)$_6$(OH)(NO$_3$)·4H$_2$O the corresponding angles are 78 and 12°.[23,52] In Zn(Im)$_6$Cl$_2$·4H$_2$O which has a less symmetrical structure, each angle is different, but all are less than 15° (and greater than 75°). Freeman has commented on the variability of imidazole bonding to metal ions.[38] There is little indication for a strong electronic restriction on the orientation of the imidazole plane relative to a square-planar or octahedral coordination geometry about a metal ion. In general, the bond distances in the complexed imidazole ring do not vary much from those in the free ligand, and Freeman has calculated average bond lengths for complexed



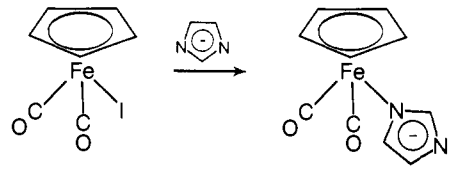
imidazole rings in imidazole and histidine derivatives.[38] The octahedral Ni(II), Cd(II), and Co(II) complexes which have been reported since these averages were calculated show no substantial deviations from these average values with the possible exception that the C–C bonds in these complexes are somewhat shorter than the earlier average with one exception in Co(Im)$_6$CO$_3$·5H$_2$O. The deviations from these values in the case of the imidazoles in Cu(Im)$_4$(CH$_3$OCH$_2$CO$_2^-$) were mentioned earlier. The imidazole bond lengths in both Zn(Im)$_6$Cl$_2$·4H$_2$O and Co(Im)$_6$CO$_3$·5H$_2$O show a somewhat larger deviation from the average than the other hexakis structures with bond length ranging from 1.28 to 1.53 Å being reported. In general there is sufficient variation among individual rings that attempts to attach structural significance to changes in bond length of 0.03 Å or less seem questionable. The source of the even larger variations noted in some of the complexes is not clear.

Imidazole effects displacement of carbon monoxide and iodide from cyclopentadienyldiiodocobalt carbonyl.[84] No complexes in which imidazole acts as a π-type ligand



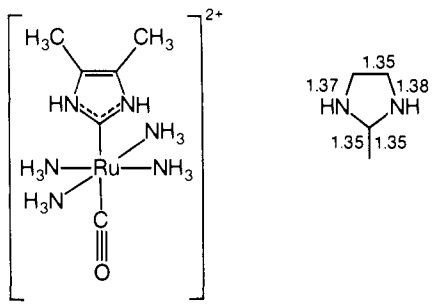
(9)

have been reported. Attempts to make "diazaferrocenes" by introducing imidazole as a π-bonded ligand have given instead products in which bonding is through the pyridine-type lone pair.[85] Monoazaferrocenes derived from pyrrole are known[86,87] but have limited stability and have not been much studied.



(10)

Recent work has established that the imidazole ring can be bound to transition metals *via* the 2-carbon atom.[43,88] Imidazole and several of its C-4 and C-5 substituted derivatives form ions of composition $[(NH_3)_4Ru(Im')X]$ with Ru(II) and Ru(III), where X can be Cl^-, H_2O, or CO. Structural conclusions indicating the carbon bound arrangement based primarily on pmr studies of the diamagnetic Ru(II) compounds have been confirmed by a crystal structure determination on the hexafluorophosphate salt of ion **5**.[43] The bond lengths for the



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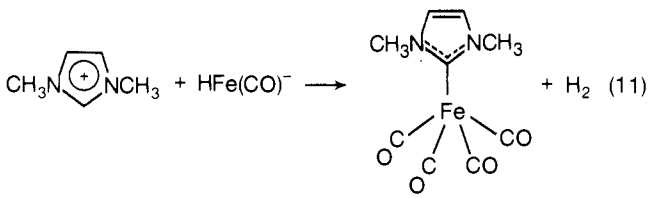
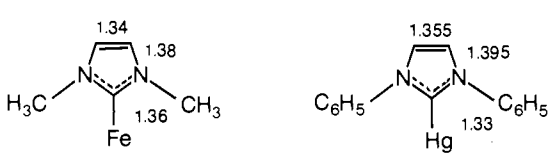
carbon-bound ring indicate some shortening of the C–C bond relative to the N-coordinated ligand but remain in the aromatic range. The ligand in such ions is the imidazolium ylide, a neutral dipolar tautomer of imidazole. The Ru(II) ion, which is a very effective π donor, may be a particularly favorable case for this mode of bonding since in the C-bonded arrangement the imidazolium can act more effectively as a π-acceptor than the N-bonded structure. Ru(II) is a low-spin d^6 transition metal ion species, as are some complexes of Fe(II) that occur in biological systems. A naturally occurring imidazolium ylide complex with low-spin Fe(II) seems a possibility.

A similar type of carbon bonding has been found in Fe(0)[89] and Cr(0)[90] carbonyl compounds (eq 11) and in



(11)

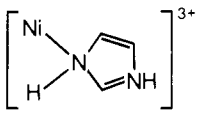
a mercuric complex derived from the 1,3-diphenylimidazolium ion.[91] Crystal structure determinations have been reported in the case of the Fe(0) and Hg(II) species.[92,93] The ring dimensions in these ions are quite



similar. The chemistry of the carbon-bound complexes remains largely unexplored at this time.

There have been several studies of the kinetics and mechanisms of the introduction of imidazole ligand into the coordination sphere of metal ions. Results for the substitution of water provided by fast reaction techniques indicate that the second-order rate constant is about 26 times greater for Co(II) than for Ni(II).[94] Similar substitution rate constants are found for nitrogen donors in other neutral molecules. The rate constants are characteristic of the metal ion rather than the ligand and reflect rate-determining loss of water from the metal ion coordination sphere.[95]

Some variation appears in the second-order rate constants for neutral imidazole substitution on aqueous Ni(II) near 25°; values of 3.2,[22] 5.0,[94] and 6.4[96] $\times 10^3$ sec^{-1} M^{-1} have been reported. Differences of this kind are not uncommon in fast reaction measurements where a large excess of metal ion exists. A more serious concern is the magnitude of the rate constant involving a term that contains the concentration of imidazolium cation, $[ImH^+]$. A value[22] of 400 sec^{-1} M^{-1} has been amended[96] to about 200 sec^{-1} M^{-1}, but since the formation constant used in the revision is too high, the amended value is probably still too high. These rate constants for the term involving $[ImH^+]$ depend on estimating a small intercept value read off a plot.[96] On the scale drawn, the intercept value may be zero. From the equilibrium and rate constants for the reverse reaction in acid solutions, a derived rate constant for substitution by ImH^+ on Ni(II) is but 5 $\times 10^{-3}$ sec^{-1} M^{-1}, a value smaller by a factor of 4 $\times 10^4$ than the 200 sec^{-1} M^{-1} considered above as an upper limit.[96] Thus the contribution of a term in $[ImH^+]$ to the rate of substitution of imidazole on Ni(II) appears to be small. It was suggested that the "nonbonded" electron pair of the pyrrole nitrogen is the site of the nucleophilic reactivity of the imidazolium cation.[22] Electrostatic repulsion and structural consider-



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ations assign a high energy to the proposed intermediate (section II) and argue against it being the correct explanation for the presence of any $[ImH^+]$ term in the rate law.

Shepherd has studied the kinetics of substitution by imidazole on $[(NH_3)_5RuOH_2]^{2+}$. There are terms for substitution both by Im and ImH^+ with k values of 0.20 M^{-1} sec^{-1} and 2.7 $\times 10^{-3}$ M^{-1} sec^{-1}, respectively, at 25° and μ = 0.10.[44] Corresponding rate constants for neutral and protonated pyridine are 0.093 and 3.1 $\times 10^{-3}$ M^{-1} sec^{-1}. Thus a term in protonated substituting ligand also appears for pyridine. The second-order rate constants expressed in terms of protonated entering ligand are not only nearly identical for the two ligands substituting into the Ru(II) complex, but also this pair of constants is close to the final estimated value for entry of imidazolium ion into aqueous Ni(II). An additional pathway for water release involving specific hydrogen ion catalysis can account for terms containing protonated ligand. The nature of the proposed pathway suggests a leveling effect among rate constants for different metal ions with similar ligands.

The kinetics of acid-catalyzed aquation of $[(NH_3)_5Im-Ru]^{2+}$ have been studied[43] and compared with similar acid mediated cleavage of ligand from $[(NH_3)_6Ru]^{2+}$ and

$[(NH_3)_5PyRu]^{2+}$.[44,97] The imidazole complex is labile in comparison with both the ammonia and pyridine analogs as illustrated by the rate data below. (The units M^{-1} refer to the concentration of replaceable ligand.) Part of

$$[(NH_3)_5RuB]^{2+} + H_3O^+ \underset{}{\overset{k_1}{\rightleftharpoons}} [(NH_3)_5RuH_2O]^{2+} + BH^+ \quad (12)$$

B	k_1, M^{-1} sec^{-1} (at μ = 1.0)
NH_3	20×10^{-5}
pyridine	5.4×10^{-5}
imidazole	3380×10^{-5}

this lability may be ascribed to the poor π-acceptor properties of imidazole since it is known that in the pyridine series the rate of aquation decreases as the pyridine is substituted by electron-withdrawing groups.[97] However, the fact that imidazole cleavage is some 170 times faster than cleavage of ammonia (no π-acceptor ability) indicates that other factors must also be in operation. The imidazole–ruthenium bond seems to be somewhat kinetically labile since imidazole is lost in preference to ammonia by at least 10:1 from $[(NH_3)_5ImRu]^{2+}$ while pyridine is eliminated from $[(NH_3)_5PyRu]^{2+}$ at only about twice the rate of ammonia.[97]

A limited amount of research has been performed on the reactivity of coordinated imidazole. Protonated imidazole may serve as a general acid catalyst and proton removal from ImH^+ by base or substitution by a metal ion are both effective in reducing general acid catalysis nearly to the vanishing point. Neutral imidazole may function as a general base catalyst or nucleophile due to the lone pair at N-3. Coordination of a metal ion at the lone pair site destroys these attributes. In the iodination of imidazole, base catalysis by imidazole itself is eliminated by its complexation with Ni(II).[98] Neutral imidazole catalysis of *p*-nitrophenylacetate hydrolysis is inhibited by the presence of coordinating metal ions such as Cu(II) and Zn(II). Careful attention to the reduction in rates provides a kinetic method for determination of formation constants between these metal ions and imidazole which agree excellently with values determined potentiometrically.[99] The potential value of the method lies in its ability to yield formation constants of metal ions bound to imidazole side chains where standard potentiometric methods may not provide sufficient selectivity. The kinetic method has been applied to evaluation of formation constants of Cu(II) and Zn(II) to the imidazole side chain of a histidine-containing tripeptide.[100]

The fusion of a benzene ring at positions 4 and 5 in benzimidazole does not perturb the electronic structure of the heterocyclic molecule dramatically. The basicity is lowered with the pK_a being 5.6 as compared to 7.1 for imidazole.[101,102] The formation constants for Cu(II) and Cu(I) have been measured in aqueous solution. In both cases the logarithm of the first formation constant is substantially smaller for benzimidazole: Cu(II), 3.5 *vs.* 4.3 for Im; Cu(I), 4.5 *vs.* 5.8 for Im. The only other stability constant data for benzimidazole pertain to organic solvents.[103]

The number of preparative and structural studies on transition metal ion complexes containing benzimidazole as a ligand is also relatively small. The maximum number of BzIm that has been found at a metal site is four. This limit is presumably imposed primarily by steric factors. Complexes of Co(II) containing one, two, and four benzimidazoles have been isolated.[104] The isolation of a substance of composition $Co(BzIm^-)_2$ indicates that benzimidazole, like imidazole, can act as a bridging ligand forming polymers when coordinated as its conjugate base. Infrared data have led to assignment of tetrahedral geometry to $Co(BzIm)_2Cl_2$ and $Co(BzIm)_2Br_2$.[105]

Nickel halides and benzimidazole form a series solid complexes of composition $Ni(BzIm)_4X_2$. These complexes are unstable in solution, losing two benzimidazole molecules. Six forms, including solvates, of both the chloride and bromide which differ in spectral and/or magnetic properties have been described, but a complete description of the structural variation among the forms is not available as yet.[106] One of three distinct forms of $Ni(BzIm)_4Cl_2$ has been subjected to X-ray structure determination.[107] The four benzimidazoles occupy octahedral sites in a binuclear cation in which one chloride bridges two Ni ions. The coordination position trans to the chloride bridge is occupied by a chloride ion. The benzimidazole rings are rotated out of the plane defined by the coordinating nitrogens minimizing steric repulsions. The structures of nickel complexes in general are sensitive to the π-acceptor and steric properties of neutral ligands and to the identity of coordinated

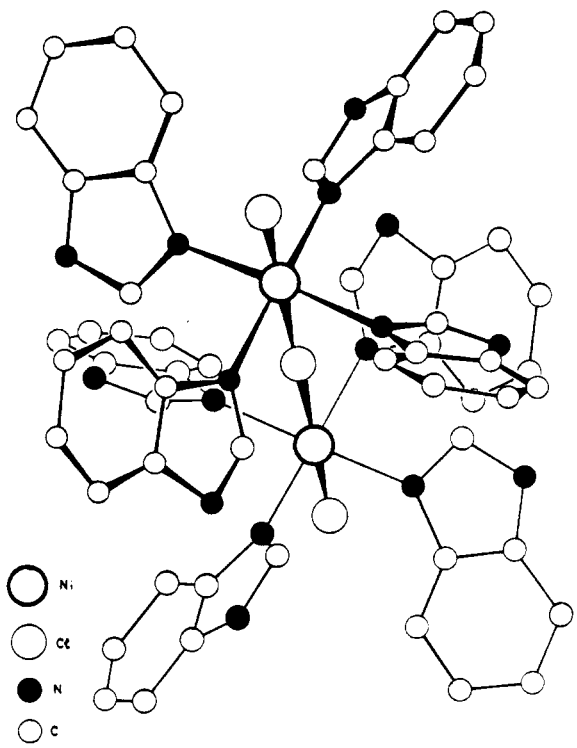


Figure 3. The $[Ni_2(BzIm)_8Cl_3]^+$ unit in μ-chloro-bis(chlorotetrabenzimidazole)nickel(II) chloride. Reproduced from ref 107.

anion.[108-110] Thus, the variety of structural types, indicated by spectral and magnetic studies of nickel complexes of benzimidazole and its substituted derivatives,[106,111,112] does not necessarily indicate any unique interaction with benzimidazole as opposed to other ligands.

Copper forms complexes of composition $Cu(BzIm)_4X_2$ containing a variety of common anions.[113,114] Also known is $Cu(BzIm^-)_2$, an insoluble material whose structure is uncertain but probably contains at least some of its Cu(II) ions in a tetrahedral environment. A strongly distorted octahedral structure is assigned to the $Cu(BzIm)_4X_2$ series for X = Br, Cl, NO_3.

A series of bis and tetrakis benzimidazole complexes of Pd(II) has been described as well as the species $Pd(BzIm^-)_2$.[115] Also reported are tetrahedral zinc complexes $Zn(BzIm)_4X_2$.[105]

Both the carbon-bound and nitrogen-bound forms of benzimidazole have been observed in the pentaammine

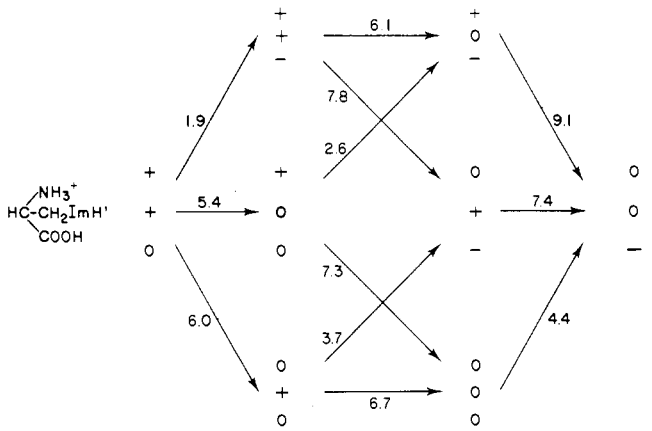


Figure 4. Histidine deprotonation scheme near 25° and 0.15 ionic strength. Signs indicate charges on ammonium, imidazole, and carboxylic acid groups, respectively. Numbers on arrows are microscopic pK_a values. Predominant deprotonation equilibria are across top of figure. Pathway starting on left with pK_a values of 5.4 and 7.3 refers to histidine methyl ester.[116] Other values found from properties of cyclic systems and electrostatic additivity[117] or estimated with consideration of imidazole propionic acid deprotonations[118] as a model.

Ru(II) and Ru(III) series.[43] The properties of carbon-bonded benzimidazole and the possible existence of this bonding mode in the case of other metal ions remain to be studied.

IV. Histidine Complexes

The importance of histidine interactions with transition metal ions in biological systems has been recognized for some time. The imidazole nitrogen of histidine residues provides one of the primary means by which metal ions may be bound to proteins. The histidine molecule presents three potential coordination sites in aqueous solution. The carboxyl group (pK_a = 1.9), the imidazole nitrogen (pK_a = 6.1), and the amino nitrogen (pK_a = 9.1) become available for complexation as pH increases. A complete ionization scheme for histidine is shown in Figure 4. The pK_a value anticipated for a histidyl residue in a polypeptide chain is shown at the bottom, center of Figure 4 where for the (0 + 0) → (0 0 0) deprotonation of the imidazolium group from an otherwise uncharged molecule, pK_a = 6.7. This value is within the range commonly observed for histidyl residues titrating normally in proteins. According to the difference of the logarithms of the microconstants 9.1–7.4 = 1.7 in Figure 4 for overall net zero charged histidine with an ionized carboxylate group, the molar concentration of the imidazole protonated species is 2% that of the ammonium protonated species at any pH.

Because they yield definitive information about structure in a crystal, the results of X-ray studies are described first. However, several instances will be mentioned where the crystal was grown in a solution where its structure represents only a minority species. The various crystal studies have shown that histidine can use each of the three potential coordination sites for bonding to metal ions. Metal ions which adopt octahedral coordination geometry can bind histidine as a terdentate ligand. Structures of bis(L-histidinato)cobalt(II)[119] and L-histidinato-D-histidinatocobalt(II)[120] have been described. Only the imidazole rings are trans in the former while the latter possesses an all-cis structure. The imidazole-N-cobalt bond lengths are in the range 2.07–2.19 Å. Racemic Ni(D-His)$_2$·Ni(L-His)$_2$ crystallizes from a solution containing both optical isomers of histidine with octahedral coordination at Ni but with only L- or D-histidine coordinated to

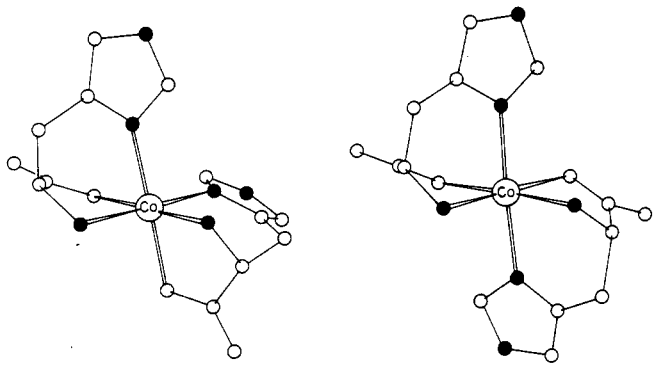


Figure 5. L-Histidinato-D-histidinatocobalt(II) (left) and bis-L-histidinatocobalt(II) (right). Reproduced from ref 120.

any individual nickel ion.[121] Again only the imidazole groups are trans. The average of six, nearly equal, metal ion–donor atom bond distances is 2.10 Å. Similarly to the case of the Ni(II) complex, crystals of Zn(His$^-$)$_2$ prepared from a solution of racemic histidine contain ligand of only one optical configuration about each Zn(II).[122] Zn(L-His$^-$)$_2$ has been crystallized from solutions of optically pure ligand.[123] An analogous Cd(L-His$^-$)$_2$ complex has also been crystallized.[124] It may be described as possessing an irregular octahedral geometry with the Cd–O bonds being about 0.25 Å longer than the Cd–N bonds. Even more irregular are the Zn(II) complexes which show an approximately tetrahedral disposition of the four nitrogen donor atoms at 2.04 Å and a much weaker interaction of the carboxylate oxygens 2.8–2.9 Å from Zn(II).

Crystallization from moderately acid (pH 3.7) solutions containing L-histidine and $CuSO_4$ in a 2:1 molar ratio with excess $NaNO_3$ affords crystals of composition Cu(His)$_2$(NO$_3$)$_2$(H$_2$O)$_2$. The ligand molecules are bound to Cu *via* trans carboxylate and amino groups. The imidazole nitrogen is not involved in coordination and is presumably protonated.[125] The coordination about Cu(II) is a typical strongly distorted octahedron with distant water molecules at the axial sites.

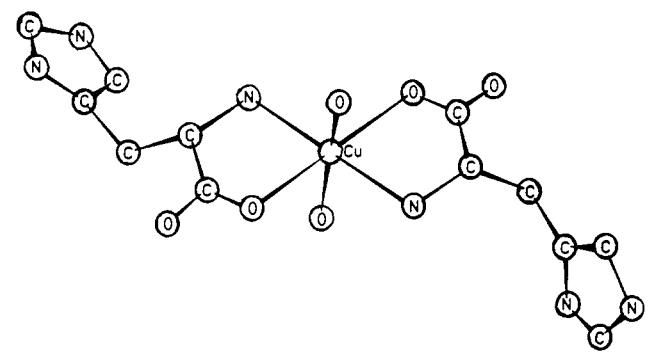


Figure 6. The bis(L-histidine)copper(II) cation in Cu(His)$_2$(NO$_3$)$_2$(H$_2$O)$_2$. Reproduced from *Acta Chem. Scand.*, **20**, 2310 (1966).

Crystals of composition Hg(His)$_2$Cl$_3$ have been obtained from Hg(II)Cl$_2$ and histidine hydrochloride. The coordination to mercury involves only a carboxylate oxygen with a Hg–O bond length of 2.54 Å.[126]

A crystal structure determination has also been done of a mixed ligand Cu(II) complex of composition Cu(His$^-$)(Thr$^-$).[127] In this crystal the imidazole nitrogen and amino nitrogen located about 2.0 Å from Cu(II) are the principal histidine coordination sites with the histidine carboxylate weakly coordinated in an irregular apical position 2.6 Å from Cu(II). On the other side of the tetrago-

TABLE V. Negative Enthalpies of Formation of $M^{II}(L\text{-}His^-)_2$[a]

Co	Ni	Cu	Zn	μ, M	Ref
	16.2	21.0	11.6	0.1	b
	16.6	21.3	11.7	0.1	c
	16.5	20.0	11.4	0.10	d
		21.3		0.16	e
9.6	13.6	22.1	8.3	0.2	f
12.3	18.9	20.1	11.8	3.0	g

[a] In kcal/mol at 25°. Except for ref f, all values were determined by calorimetry. [b] A. C. R. Thornton and H. A. Skinner, *Trans. Faraday Soc.*, **65**, 2044 (1969). [c] W. F. Stack and H. A. Skinner, *ibid.*, **63**, 1136 (1967). [d] Reference 159. [e] J. L. Meyer and J. E. Bauman, Jr., *J. Amer. Chem. Soc.*, **92**, 4210 (1970). [f] E. V. Raju and H. B. Mathur, *J. Inorg. Nucl. Chem.*, **31**, 425 (1969). [g] D. R. Williams, *J. Chem. Soc. A*, 1550 (1970), and ref 136.

nal plane threonine is bound as a substituted glycine with its amino and carboxylate donor atoms 2.0 Å from the Cu(II). A water oxygen occupies the sixth coordination position about Cu(II). Attempts to prepare crystals of $Cu(His^-)_2$ suitable for X-ray analysis have so far been unsuccessful, but the project remains a worthy goal. The crystal data thus demonstrate that a terdentate ligand and bidentate chelation using either an imidazole-amino pair or an amino-carboxylate pair are feasible. The third possible bidentate combination, imidazole–carboxylate, would be disfavored by the seven-membered ring involved, and an example of such chelation in a crystalline structure is lacking, but this bonding mode has been considered in the case of solution species.

The crystal structure of $(histamine)_2Cu(ClO_4)_2$ has also been determined.[128] No unusual structural features were revealed. Four nitrogen donors occur in the coordination plane with two axial perchlorates. The nitrogen donor atoms from two planar imidazole rings occupy trans coordination positions 1.98 Å from the Cu(II). The structure of the nickel complex has also been determined.[129] It is octahedral, with water and an oxygen from perchlorate completing the coordination sphere.

The X-ray results can provide useful information on the types of complexes apt to be present in solution but clearly are incapable of providing definitive solution structures. Often the types of results accessible on the solution are subject to more than one interpretation. This has been true for the case of histidine complexes, especially with regard to Cu(II). One approach to elucidation of the solution structure has relied upon comparison of stability constants and titration behavior with known systems.

Stability constants for histidine and a variety of divalent metal ions appear in Table II (section III). Both the stability constants and the enthalpies of formation collected in Table V follow the usual stability order.[39] Enthalpies for metal ions other than those included in Table V appear in some of the references to the table, but their weak binding results in poorer agreement among different investigators. For the case of Cu(II) which is discussed below, the enthalpy values dominate the free energy with the result that similar arguments may be made with either quantity. Comparison with other compounds indicates that the large negative enthalpies of formation result from coordination of Cu(II) with nitrogen donors; heats for coordination with oxygen donors are small. From such comparisons coordination of four nitrogen donors is indicated in $Cu(His^-)_2$, but as suggested below, they are not coplanar.

Copper(II) coordination compounds typically consist of four nearby donor atoms disposed in an approximate plane about the metal ion with the possibility of one or two more distant axial ligands. The three donor atoms of terdentate anionic histidine cannot all occupy planar positions simultaneously, and the question arises as to which occupies the axial positions about Cu(II). Complexes of terdentate anionic histidine dominate above pH 7 and at lower pH several kinds of protonated ligand species may be chelated in bidentate modes. The composition of the predominant protonated species and their structures have been matters of uncertainty.

In order to elaborate the structures of complexes in solution, it is first necessary to determine the frequency with which a species of a given composition occurs and second to establish the structure(s) of each species of set composition. The first point on the compositions of solution complexes may often be solved by detailed computer-assisted interpretation of titration results over a range of conditions. Elucidation of the structures of the complexes requires inputs from other physical properties. These two points will be reviewed for the Cu(II)–histidine system. For convenience we designate anionic histidine by A^- and Cu(II) by M^{2+}.

The composition of the predominant complexes of histidine and Cu(II) in solution depends upon the ligand to metal ion ratio, total concentration, and pH. For a 1:1 ratio the MA^+ species dominates at pH 6 being converted to $MA(OH)^0$ above this pH with $pK_a = 8.0$. Below pH 5 no species comprises more than 50% of all Cu(II), the greatest contributor being MAH^{2+} which maximizes near pH 3.7 at less than 40%. Most of the remaining ligand is unbound at pH $\leq$ 3.7. These conclusions and the contributions of several minor species in 1:1 solutions have been portrayed graphically.[130]

Solutions containing 2:1 or higher molar ratios of histidine to Cu(II) possess similar species distribution at most pH values.[131] Nearly 100% of the Cu(II) is bound as MA_2^0 from pH 7 to 8. At higher pH, species with one less proton due either to addition of OH^- or pyrrole hydrogen ionization (section IX) begin to appear. The species MA_2H^+ occurs to the extent of 90% from pH 4 to 4.5 and is converted to MA_2^0 with $pK_a = 5.5$. The only other species with a frequency approaching 50% is that of MAH^{2+} just below pH 3. In 2:1 solutions the maximum amount of MA^+ occurs just above pH 3 where about 25% of the Cu(II) is so bound. At higher histidine to Cu(II) ratios this percentage falls to 10% or less. The species of composition $MA_2H_2^{2+}$, corresponding to that on which the X-ray structure was performed (see above), seems never to represent more than 15% of the Cu(II) and even near pH 3.3, where it occurs in maximum percentage, is exceeded by the amounts of both MAH^{2+} and MA_2H^+. Thus the crystals for the X-ray analysis were prepared from a solution that contains less than one-fifth of the Cu(II) in the form of the precipitated ion and in which two other species are more prevalent. The results described in this paragraph for the distribution of complexed species as a function of pH in solutions containing 2:1 and greater mole ratios of histidine and Cu(II) have recently been displayed graphically.[131a]

Results of the detailed titration studies[130,131] indicate that the 1:1 and 2:1 solutions of histidine and Cu(II) contain as major species MA^+, MAH^{2+}, MA_2H^+, and MA_2^0. Relationships among these species are summarized in Figure 7. In attempting to establish a structure for each species, we appreciate first that a single species may possess more than one structure. As a result the structures suggested as predominant are not apt to be the only ones present in solution. Indeed, the number of different structures suggested in the literature for some of the species is in itself a kind of evidence for the presence of more than one structure in solution. Though there

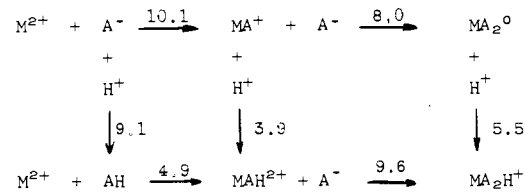


Figure 7. Logarithms of equilibrium formation constants in Cu(II)–histidine solutions at 25° and 0.1 ionic strength.[131] Cu(II) is represented by M^{2+} and histidine anion by A^-.

have been exceptions,[132,133] we assume that a basic amino or imidazole nitrogen on a coordinated histidine will not remain free but be bound to either metal or hydrogen ion.

It seems to be generally agreed that the species MA^+ consists of a terdentate histidine with two nitrogen donor atoms in the coordination plane with an axial carboxylate group. That the carboxylate group is only weakly bound is suggested both by a stability constant comparison[134] and the irregular geometry of the histidine carboxylate group in the complex with threonine.[127]

The location of the proton in MAH^{2+} is more controversial. Attachment at the amino groups with imidazole–carboxylate chelation has been the most popular view,[40,130,135-137] but a protonated imidazole group with chelation as a substituted glycine has also been suggested.[134] Infrared spectra have been interpreted as involving imidazole involvement in Cu(II) binding.[40] However, several conclusions of this infrared study of 1:1 solution complexes of divalent metal ions and histidine disagree with results of other physical methods. For the case of the 1:1 Cu(II)–histidine complex the change to MA^+ as the dominant species above pH 4 was not recognized.

The stability constant shown in Figure 7 for formation of the Cu(II) complex with ammonium protonated histidine, AH, is log K = 4.9. It does not seem possible to reconcile this value with Cu(II) coordination at the basic imidazole and carboxylate sites. Imidazole-4(5)-propionate with pK_a = 7.56 and log K_1 = 4.6 for Cu(II)[118] is slightly more basic than imidazole (Table II) accounting entirely for its slightly greater stability constant. It appears that the carboxylate group contributes little to the stability of the Cu(II) complex and that chelation is weak.[134] The imidazole group of imidazole-4(5)-propionate is 30 times more basic than that of histidine so that the stability constant log K = 4.9 for protonated histidine seems at least ten times too large to be assigned exclusively to Cu(II) coordination at the imidazole–carboxylate locus. On the other hand, transfer of the proton from the ammonium group to the imidazole group of histidine (9.1–7.4 = 1.7 log units from Figure 4) and chelation of Cu(II) at the substituted glycine mode yields a stability constant log K = 4.9 + 1.7 = 6.6. This value for Cu(II) chelation at the substituted glycine locus of histidine of pK_a = 7.8, with a protonated imidazolium group, is consistent with log K_1 = 8.1 for Cu(II) chelation with glycine of pK_a = 9.7. This interpretation of the structure of MAH^{2+} is consistent with the X-ray structure of $MA_2H_2^+$ where only glycine coordination of histidine to Cu(II) occurs.[125] Because of nearly compensating heats of complexation and deprotonation, enthalpy values do not distinguish between alternative nitrogen–oxygen chelating loci of Cu(II) to monoprotonated histidine. A recent determination of the stability constant for coordination of Cu(II) with *N*-trimethylhistidine (pK_a = 6.0) yields log K_1 = 3.6.[137a] This result supports the preceding arguments by indicating weak or no carboxylate chelation and the incorrectness of assigning log K = 4.9 in protonated histidine exclusively to imidazole coordination.

The solution structure of the third species, MA_2H^+, is considered to be a combination of the two already mentioned with one terdentate histidine with a weakly coordinated apical carboxylate and one bidentate histidine chelated in the tetragonal plane in a glycine-like mode with a protonated imidazolium group. The visible absorption and mainly positive circular dichroism spectra of the monoprotonated 2:1 L-histidine complex, MA_2H^+, near pH 5 is similar to that of the mixed complex with L-histidine and L-1-methylhistidine, a bidentate ligand with a nonchelatable imidazole group.[138] Further support for this mixed histamine-like and glycine-like bonding mode in the tetragonal plane is mentioned in section V. The pK_a values of 3.9 and 5.5 (Figure 7) for MAH^{2+} and MA_2H^+, respectively, refer primarily then to imidazolium deprotonations that are promoted by complexation with Cu(II) to yield MA^+ and MA_2^0.

Because it is dominant in neutral and basic solutions containing at least a 2:1 molar ratio of histidine to Cu(II), the structure of the species MA_2^0 has received the most attention. As virtually every conceivable structure has been suggested, it is not feasible to review the history here. Examples occur where profound reasoning has led to unlikely structures and also where faulty interpretation has produced probable structures. In the following discussion the emphasis is on the salient experimental facts that any currently proposed structure must account for.

The difference between the logarithms of the first and second formation constants, log K_1 − log K_2, for Cu(II) and several ligands is intermediate for histidine, Δ log K = 2.1, between those of histidine methyl ester, histidinol,[134] and histamine, Δ log K = 2.6–3.0 (Table II), and those for amino acids that chelate as substituted glycines, Δ log K = 0.8–1.4. This result suggests that in the 2:1 complex with Cu(II) the two histidines do not chelate in identical histamine-like or glycine-like modes.[134,139] In addition the high negative enthalpy of formation of the 2:1 complex indicates that all four nitrogens are coordinated. A stability constant comparison with histidinol suggests that the carboxylate group contributes little to the stability of a 1:1 histidine:Cu(II) complex and is weakly coordinated.[134]

Further evidence regarding the structure of the MA_2^0-Cu(II) complex of histidine is provided by visible absorption and circular dichroism (CD) spectra. The absorption maximum occurs at a relatively long 640 nm, suggesting apical chelation by a group of greater ligand field strength than water.[133,134] Optically active L-amino acids chelated in a glycine-like mode yield net negative CD through the d–d absorption region of transition metal ions including Cu(II).[140,140a] This typical net negative CD is also observed for the 2:1 Cu(II) chelate of L-1-methylhistidinate where the methyl group blocks the imidazole nitrogen that is otherwise capable of chelation.[133,134] The 2:1 Cu(II) complexes of anionic L-histidine yield a positive visible CD, as do those of L-2,4-diaminobutyrate and asparagine dianion.[134] Solutions containing equimolar mixtures of Cu(II), anionic L-histidine, and one bidentate ligand such as histamine or L-1-methylhistidinate yield appreciable negative CD at 600 nm, indicating that they do not represent the behavior of a second mole of anionic histidine in a 2:1 solution.[134,138] These results suggest that all three donor atoms on the second histidine anion are involved in bonding.

The predominant structure for $Cu(L\text{-}His^-)_2$ that seems to fit best all these results is one where both L-histidine molecules are terdentate with trans amino groups. Two coordination planes contain four donor atoms from two amino, one imidazole, and one carboxylate group, lead-

ing to two identical sets of oxygen and imidazole nitrogen axial donors. Thus the structural flexibility that seems to be required appears within a single complex. This structure also possesses an approximate plane that contains two imidazole nitrogen and two carboxylate oxygen donors. The pronounced tendency for unsaturated nitrogen donors to prefer mixed coordination with oxygen donors is reviewed in section V. For this and other reasons this structure seems to be preferred to the one with trans imidazole groups. The third possibility, the structure with trans carboxylate groups, appears to provide fewer advantages compared to the other two in accounting for the experimental results.

Other physical methods have also been employed in attempts to ascertain the structures of metal ion complexes with histidine. Two papers report the results of electron spin resonance spectra on frozen solutions at liquid nitrogen temperature containing a 10:1 molar ratio of histidine to Cu(II). Because there was no significant change in an esr parameter from pH 3 to 8, the first study concludes that only one major species is present.[141] This conclusion is not supported by the authors' reported visible absorption spectra which give maxima from 625 to 690 nm nor by the titration studies[131,135,136] which indicate three different predominant species throughout the pH range. In the second study, interpretation of esr spectra of frozen 0.1 *N* NaOH solutions of histidine, glycine, histamine, and mixtures of the last two ligands with Cu(II) was combined with results from other physical measurements to suggest a mixed ligand structure for $Cu(His^-)_2$ that is identical with that described above with trans amino groups.[142] However, because of the high basicity of these solutions, most of the complexed histidine molecules should have undergone pyrrole hydrogen ionization[143] (section IX).

The rates of complexation of Cu(II) with neutral histidine[12] and of Ni(II) with histidine and some derivatives[22,96] have been measured. An important conclusion from the Cu(II) study is that the rate of reaction with neutral histidine is sterically controlled; the rate of reentry of water in place of one ligand donor atom in a primary coordination sphere is greater than the rate of coordination of the second ligand donor on a chelatable ligand. We shall utilize this conclusion for Cu(II) and histidine in the following discussion on selective broadening of ligand resonances in pmr spectroscopy.

Broadening of ligand lines in pmr spectroscopy has been utilized in attempts to delineate the modes of Cu(II) binding to histidine and derivatives.[137,139] Compared to Co(II), where the relatively short electron spin relaxation time permits observation of ligand resonances at stoichiometric concentrations,[33] the long electron spin relaxation time for Cu(II) usually results in severe broadening of ligand resonances so that experiments are performed at ligand to Cu(II) mole ratios of 10^3 or greater. The predominant mode of complexation at these extraordinary ligand to metal ion ratios may not be the same as those occurring at stoichiometric concentrations. Since the rate of reaction of Cu(II) with histidine is sterically controlled,[12] in a selective broadening experiment Cu(II) can be expected to interact in only a monodentate mode before it rapidly passes to the next histidine molecule. An implication in application of a selective broadening experiment is that the distance dependence of a broadened resonance varies as r^{-6} according to the dipolar mechanism. This result does not seem to have been established for Cu(II) interactions with imidazole and derivatives, and indeed there is suggestive evidence that a scalar (contact) mechanism prevails for line broadening. If the scalar interaction occurs *via* the π-electron system, the unpaired electron spin density, which may give rise to broadening, is distributed according to electronic properties of the orbitals and not upon distance from the metal ion. Since it is flanked by the two nitrogens, the H-2 of imidazole derivatives must be at least as close to a coordinated metal ion as H-4, yet greater broadening is observed in the latter resonance when Cu(II) is added to solutions containing imidazole and ten derivatives including histidine.[134,144] Further support for significant contributions to broadening from a scalar mechanism comes from the relatively large band width increment observed for Cu(II) with 3-methylhistidine.[139] These pitfalls render equivocal any current interpretation of Cu(II) selective broadening experiments to modes of binding in histidine at stoichiometric concentrations.

The course of changes with pH in the structures of the complexes present in a solution containing a 2:1 molar ratio of histidine to paramagnetic Co(II) was deduced from contact shifts observed in proton magnetic resonance spectra.[33] Below pH 1 only the HDO peak is shifted as Co(II) is ineffective in competing with protons for basic sites on histidine. From pH 1 to 3.5, shifts of the aliphatic protons are attributed to some Co(II) bonding at the carboxylate group. The shifted HDO peak begins about pH 3 to move to a position free of Co(II) which is complete about pH 6 where no water remains in the coordination octahedron. One set of strongly shifted histidine peaks occurs from pH 4.5 to 7, and a second set from pH 5 to 10.5. Observation of separate sets of signals indicates relatively slow exchange, and these two sets have been assigned to $Co(His^-)^+$ and $Co(His^-)_2$ complexes of terdentate histidine.[145] That the former complex is $Co(His^-)^+$ and not $Co(His^-)(HisH)^+$ is supported by a detailed potentiometric study which indicates that, in contrast to Cu(II), protonated complexes are unimportant in solutions of histidine and Co(II).[130] Finally, a new set of pmr peaks and a deep blue color appear at pH 11.5–12. This high pH complex is attributed to tetrahedral Co(II) complexed to two amino and two imidazole nitrogen donor atoms from two dianionic histidine molecules that have undergone pyrrole hydrogen ionization.[33] These ionizations take place with an average $pK_a \sim 12$ according to a spectrophotometric investigation which supports the mononuclear tetrahedral complex formulation at pH >13.5[143] (section IX).

Paramagnetic Ni(II) complexes of histidine also give rise to contact shifts in pmr spectra.[146] Diamagnetic metal ion complexes of histidine and other ligands display upfield pmr shifts when compared with spectra obtained for unbound but protonated ligand of the same net charge as the complex.[147]

Numerous papers containing information on absorption spectra of histidine complexes have been published. Earlier measurements of optical activity by optical rotatory dispersion, as well as many of their interpretations, have been superseded by more recent research utilizing circular dichroism (CD). In the ultraviolet region charge transfer transitions occur for the Cu(II) and Ni(II) complexes, and their absorption and CD have been recorded.[134,148] Solution CD or polarized crystal spectra through the ligand field bands have been reported for the histidine complexes of Co(II),[149] Co(III),[150–152] Ni(II),[153,154] Cu(II),[133,134] and Pd(II).[155,156]

Stereoselectivity has been demonstrated in metal ion complexes of histidine. In principle, a complex of a metal ion and an L ligand interacts differentially with any other L,D ligand pair in forming a 2:1 complex. The formulation is simplified when both ligands are the same chemical

TABLE VI. Mole Per Cent of Mixed L,D 2:1 Complexes in Solutions of Racemic Histidine[a]

Divalent metal ion	Mole %	Ref
Ni	72, 70	157, 158
Co	62, 63	157, 158
Zn	62	157
Cd	53	157
Cu	50	157
Statistical	50	

[a] Determined by potentiometric titration at 25° and 0.1 ionic strength.

species so that a comparison is made of an MLL or MDD complex with the MLD one.[157] The question is whether a difference in stabilities or other properties is detectable. Five methods have been applied successfully for recognition of stereoselectivity in metal ion complexes of histidine: potentiometric titrations, pmr spectroscopy, reaction rates, absorption spectrophotometry, and calorimetry.

Unlike the mixed complex MDL, the 2:1 complexes of two like ligands can dissociate in two different ways to yield a specified 1:1 complex. As a result when a solution contains equal amounts of each enantiomer, the statistical distribution of complexes is 50% MDL and 25% each MDD and MLL. The results from stability constant determinations for several histidine complexes of divalent metal ions determined for L,L- and L,D-histidine are shown in Table VI. There is excellent agreement between two independent studies for the Ni(II) and Co(II) complexes.[157,158] In addition, analysis of peak heights of each diastereoisomer in the pmr spectra of racemic histidine and Co(II) also yields 62% mixed complex.[33]

These results stand in interesting opposition to those of the X-ray structure determinations on crystals prepared from solutions containing racemic histidine. Crystals of both the Ni(II)[121] and Zn(II)[122] complexes contained equal amounts of the two unmixed complexes, ML_2 and MD_2. In contrast, results of the quantitative potentiometric studies collected in Table VI show that the mixed complex, MDL, is favored for both metal ions to an even greater extent than the 50% expected on statistical grounds. The fact that the crystals contain species that represent less than one-fifth of the complexes in solution points up again the necessity of performing experiments on solutions if information concerning the distribution of complexes in solution is desired.

Within experimental error, calorimetric measurements indicate that most of the excess free energy of 0.53 and 0.29 kcal in favor of the mixed complexes MDL of Ni(II) and Zn(II), respectively, is accounted for by the enthalpy change with only a small or zero entropy contribution.[159] The calorimetric study also reports a small excess enthalpy of 0.2 kcal in favor of the optically pure 2:1 complexes in the case of Cu(II). Since the more sensitive potentiometric titrations uncovered little stereoselectivity for Cu(II),[157] the enthalpy and entropy changes evidently offset each other in this case.

A kinetic stereoselectivity has been observed in hydrolysis of histidine methyl ester complexed to Ni(His^-).[160] The hydrolysis rate is 40% faster when histidine and its methyl ester are of opposite configurations. No stereoselectivity was observed in similar experiments when Cu(II) replaced Ni(II).[161] Transition metal ions are much less effective in promoting hydrolysis of histidine methyl ester compared to glycine esters.[116,162] For example, hydroxide ion attack on Cu(glycine ethyl ester)$^{2+}$ is about 400 times faster than on Cu(histidine methyl ester)$_2^{2+}$. In the case of glycine esters coordination at the amino group leads to significant chelation at the carbonyl oxygen, assisting hydroxide ion attack and leading to superacid catalysis by the metal ion.[116,162] The carbonyl oxygen of the already bidentate histidine methyl ester interacts directly with the metal ion much less frequently. However, a molecule of histidine and one of its methyl ester can both be terdentate in a mixed MDL all-trans complex while the ester carbonyl oxygen is sterically unable to interact directly with the metal ion in those MLL complexes that retain a trans disposition of imidazole and amino groups.

Studies with a variety of labile transition metal ion and amino acid complexes suggest that stereoselectivity is favored when a terdendate ligand with a bulky side chain is chelated to a small metal ion.[157] Stereoselectivity reported in the equilibrium and transition state free energies of Ni(II), Co(II), and Cd(II) complexes of histidine and its methyl ester has been rationalized by ascribing greater stability to the all-trans MDL isomer. Only one set of groups may be trans in the three MLL isomers. If this explanation is to apply to Zn(II), the complex in solution must be more octahedral than the nearly tetrahedral Zn(His^-)$_2$ found in the crystals. The difference of 1.1 log units for log K_1 − log K_2 for the histidine complexes of Zn(II) and Cd(II) corresponds to the 1.2 log units found for the hexacoordinate complex of histidine methyl ester with Cd(II) rather than to the appreciably smaller difference of 0.26 log unit reported for the tetracoordinate complex of histidine methyl ester with Zn(II) (Table II). This comparison suggests that the 2:1 histidine complex of Zn(II) possesses a significant amount of hexacoordinate character in solution.

Bis octahedral complexes of terdentate histidine can exist in three isomeric forms for optically pure ligand, one set of three identical groups trans in each case, and in two forms with racemic ligand, all trans and all cis, with the latter possessing twice the statistical weight. The distribution of the isomeric forms within the optically pure or racemic cases is unknown for the labile solution complexes that have been reviewed to now. All three differently colored geometric isomers of inert [Co^{III}(L-His^-)$_2$]$^+$ have been obtained by ion exchange chromatography[163] and the structures assigned on the basis of their electronic spectra.[164] Oxygenation of a solution containing slightly more than a 2:1 ratio of L-histidine to Co(II) over charcoal at 75° yields 13:3:1 mole ratios assigned to the trans imidazole, trans carboxylate, and trans amino isomers, respectively.[150] Similar treatment of a solution containing D,L-histidine yields about 90% of the product as the all-cis MDL isomer, despite the fact that the two mirror image forms represent only two of the nine possible isomers. If this distribution is thermodynamically and not kinetically controlled, it may force a reevaluation of the source of stereoselectivity in the labile octahedral metal ion complexes.

There are only a limited number of examples upon which to draw inferences concerning stability in octahedral 2:1 complexes of histidine. All cis MDL isomers occur for Co(III)[150] as just discussed and for the Co(II) complex[120] reviewed at the beginning of this section. In addition the Ni(gly$^-$)$_2$Im$_2$ complex[165] mentioned at the beginning of the next section also displays an all-cis disposition of donor groups. No all-trans examples appear to have been characterized. Only two structures are known for octahedral ML_2 systems: both the Co(II)[119] and Ni(II)[121] complexes contain trans imidazole rings. The common features of the five structures encompassing both MDL and ML_2 systems are cis oxygen and cis amino nitrogen donor atoms. If more examples of these

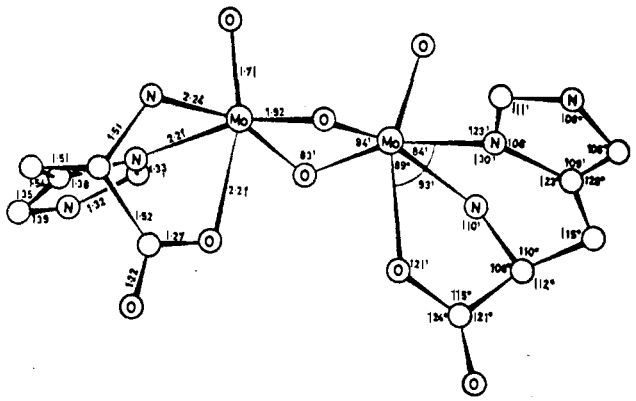
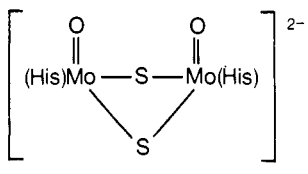


Figure 8. Molecular structure of Mo_2O_4(L-histidine)$_2$. Reproduced from ref 169.

results become established in the future and are applicable to labile octahedral complexes in solution, then the stereoselectivity observed in solutions in favor of the MDL species may be ascribed to the special stability of its all-cis isomer, while for the ML_2 species the most stable isomer appears to be trans imidazole. On this view, observation of stereoselectivity in bis histidine complexes and not in the bis complexes of diaminopropionate and aspartate is due primarily to electronic effects as the near identity of two of the three donor atoms in the last two ligands reduces the distinguishing features of the isomers to the vanishing point.

The nmr spectra of tetragonal, presumed *cis*- and *trans*-Pt(His)$_2$ have been described in both strongly acidic and strongly basic solution.[166] The conformation of the complex in both pH ranges is discussed in terms of coordination involving the imidazole nitrogen and amino nitrogen. The low pH species (pH 1) has the composition $[Pt(His)_2]^{2+}$, and it is assumed that the carboxyl group is un-ionized. At pH 12 the composition is considered to be $[Pt(His^{2-})_2]^{2-}$ with protons having been removed from the carbonyl and pyrrole nitrogen. Changes in the nmr spectra of the complexes with pH are considered to be the result of conformational changes rather than changes in the identity of the coordinating groups.

Histidine forms a complex with Mo(VI) which is diamagnetic and, therefore, presumably dimerized.[167] A weaker complex of Mo(V) has also been demonstrated to exist in solution[167] and has subsequently been isolated.[168] X-Ray crystallography[169] has established that the complex has the oxygen-bridged structure shown in Figure 8, although initial investigation of the Mo(VI) complex in solution found no evidence indicating coordination by the carboxylate, and thus the sixth coordination position in solution may be occupied by water. The Mo(V) complex reacts with hydrogen sulfide to give a new species in which it is proposed that the bridging oxygens have been replaced by sulfur atoms.[170] Although no direct evidence of histidine–molybdenum binding in enzymes seems to have been reported to date, the possibility is a subject of current study in view of the involvement of molybdenum in several enzymes which catalyze redox processes,[171] especially nitrogen reduction.



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Histidine reacts with $[(NH_3)_5RuH_2O]^{2+}$ to give initially the complex bound through the imidazole nitrogen as concluded from electronic spectral data.[172] This complex displays the same linkage isomerism to a carbon bound tetraammineruthenium complex at pH below 2[172] that is exhibited by imidazole and its simple alkyl derivatives.[43,88]

Lanthanide ion complexes of histidine have been investigated by titration, calorimetry, and pmr spectroscopy. A titration study suggests hydrolyses of Ln(III) at pH >7 even in the presence of excess histidine, which forms only weak complexes.[173] This conclusion disagrees with a titration and calorimetry study performed in 3 *M* $NaClO_4$, where though hydrolyzed complexes were sought, they are reported to be absent from pH 6 to 10.[174] Dipolar shifts of histidine hydrogens observed in the pmr spectra of solutions containing paramagnetic Nd(III) have been reported from pH 0 to 7. A weak monodentate complex with histidine bound through the carboxylate group is indicated at pH <5.[175]

It has been claimed that Be(II) chelates to histidine and derivatives *via* nitrogen donor atoms and forms complexes of strength comparable to those of transition metal ions.[176] However, the formation curves are irregular, and no evidence is offered to substantiate Be(II) complexation at the amino group in the studied pH range 4–7 where hydrolysis of complexes and aqueous Be(II) occurs.

V. Mixed Complexes of Imidazole and Histidine

In recent years it has become increasingly apparent that several transition metal ions favor coordination with mixed ligand systems to a greater extent than would be expected on a statistical basis. Sometimes this tendency to mixed complex formation is pronounced: as will be reviewed below, instead of the statistical 50%, more than 90% of a solution containing equimolar amounts of Cu(II), histamine, and serine exists as a ternary mixed complex. Mixed complex systems are also important physiologically apart from the protein systems reviewed in section VI. Of the amino acid bound Cu(II) in human blood plasma, more than 98% occurs in histidine complexes, and most of this amount is in mixed complexes with other amino acid ligands.[177]

Most of the information on mixed ligand complexes of imidazole and derivatives with transition metal ions has been gained by potentiometric solution investigations. In keeping with the format of the other sections, however, we shall review first the results of X-ray crystal structure determinations and preparations of some other solid complexes. The crystal structure of the mixed ligand complex Cu(His$^-$)(Thr$^-$)[127] was mentioned near the beginning of the last section.

A number of metal ion complexes have been prepared in which one or more of the coordinating sites is occupied by imidazole and the remainder by amino acid or peptide groups.[83,178,179] Crystal structures of some of these have been determined. These include [Cu(Gly-Gly-H)(Im)$_2$]ClO_4, Cu(Gly-Gly)(Im)$(H_2O)\cdot 1.5H_2O$ and [Cu(Gly-Gly-GlyH)(Im)(H_2O)]H_2O.[180] A common feature of the structures is an apparent tendency for the imidazole ring to be coplanar with the other three tightly held donor atoms. The ring rotates from coplanarity, however, if relief of significant steric stress can be achieved. The Cu–N bond lengths are all near 1.95 Å, and no correlation with the angle of rotation was noted.

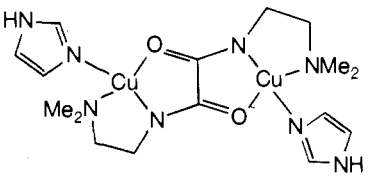
The crystal structure of Ni(Gly)$_2$(Im)$_2$ has also been determined.[165] The coordination is approximately octahedral with two imidazoles, two amino groups, and two oxy-

gens all occupying cis positions. Hence this structure is analogous to the all-cis MDL histidine systems discussed at the end of the previous section IV. One imidazole is essentially coplanar with the Ni and three other ligand atoms, but the second imidazole is rotated from the planes defined by atoms directly bound to nickel. Models indicate that steric factors restrict the imidazoles to these particular orientations.

The existence of a mixed ligand Cu(II) complex with glycylglycine and imidazole was first deduced from solution data.[178] As confirmed by the X-ray structure, this is an example of a system in which an amide linkage has been deprotonated in the process of chelation. As will be discussed more extensively in section VI, this is an important facet of peptide complexes with Cu(II) and certain other transition metal ions. At pH values where deprotonation does not occur, glycylglycine forms a bidentate chelate using the terminal amino and carbonyl oxygen atoms.[180] The carboxyl is linked to a second Cu(II) in the crystal. The fourth planar coordination site is then occupied by a second imidazole in this complex. The bidentate and terdentate bonding arrangements found in the mixed glycylglycine–imidazole complexes of Cu(II) are shown in Figure 9.

For a complex which analyzed as $Cu_2(Gly\text{-}Gly)_2\text{-}Im\cdot 8H_2O$, a structure with anionic dipeptide and bridging imidazolate and hydroxide groups was suggested.[83] It is unlikely that pyrrole hydrogen ionization (section IX) occurs before dipeptide amide hydrogen ionization in Cu(II) complexes, and the structure seems sterically improbable as well.

Imidazole also complexes with Cu(II) in a binuclear complex in which the other binding sites are an amino group and a deprotonated amide[181] from *N,N'*-bis(2-dimethylamino)oxamide. A characteristic ultraviolet absorption band of this complex has been interpreted as in-



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dicating electronic interaction between the Cu ions of the binuclear complex.

In solution the relative stability of mixed ligand complexes may be quantitatively characterized by considering only those complexes that are involved in their formation. For coordination of two ligands A and B with metal ion M we may write (excluding charges) the following equations and associated equilibrium constants.

$$\begin{aligned} M + A &\rightleftharpoons MA & K_A &= [MA]/[M][A] \\ M + B &\rightleftharpoons MB & K_B &= [MB]/[M][B] \\ MA + B &\rightleftharpoons MAB & K_{AB} &= [MAB]/[MA][B] \\ MB + A &\rightleftharpoons MBA & K_{BA} &= [MBA]/[MB][A] \end{aligned} \quad (13)$$

For labile systems in solution the mixed complexes MAB and MBA are identical, and from the properties of a cyclic system we define

$$\Delta \log K = \log K_A - \log K_{BA} = \log K_B - \log K_{AB} \quad (14)$$

The last equation also represents the equilibrium constant for the reaction

$$M + MAB \rightleftharpoons MA + MB \quad (15)$$

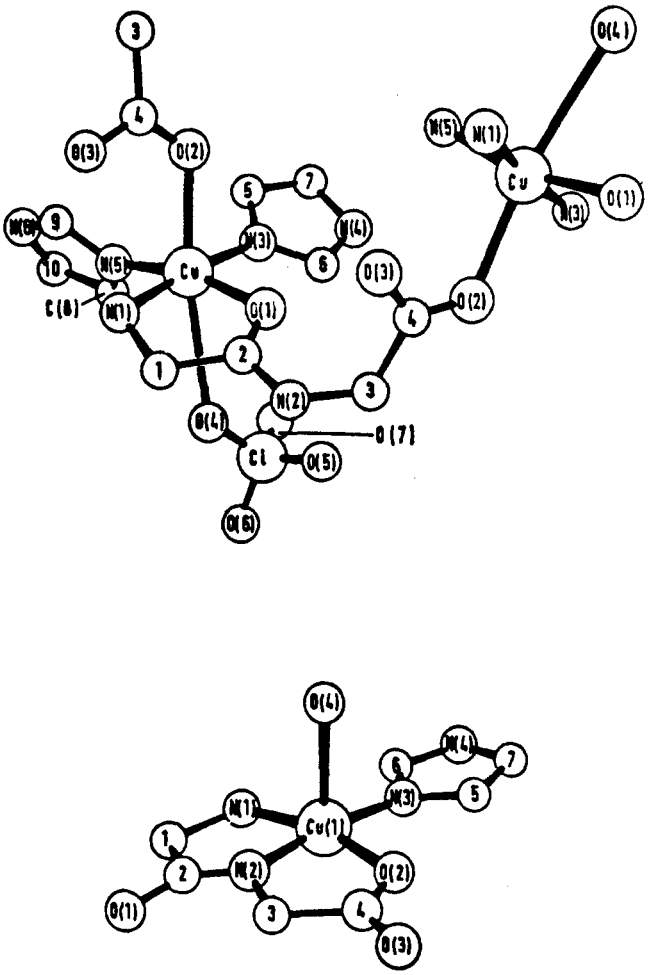


Figure 9. Bidentate and terdentate bonding of glycylglycine in mixed Cu(II) complexes with imidazole: (top) bidentate bonding in $[Cu(Im)_2Gly\text{-}Gly]ClO_4$; (bottom) terdentate bonding in $[Cu(Im)(H_2O)Gly\text{-}Gly]\cdot 1.5H_2O$. Reproduced from ref 180.

Because more coordination positions are available on aqueous metal ion M than on complex MA, we might expect ligand B to bind more avidly to M than MA, yielding positive values for $\Delta \log K$. A positive value of $\Delta \log K$ implies that the two ternary complexes with water as the second ligand on the right side of eq 15 are favored over the ternary complex MAB and the binary complex M(aq) on the left-hand side. Small positive or negative values of $\Delta \log K$ are interpreted as especially favoring the mixed ternary complex MAB.

For two identical ligands eq 15 becomes

$$M + MA_2 \rightleftharpoons 2MA \quad (16)$$

with the logarithm of the equilibrium constant given by

$$\Delta \log K = \log K_1 - \log K_2 \quad (17)$$

where K_1 and K_2 are the first and second stability constants, respectively. Extensive tabulations of these constants provide a fund of information on the tendency for mixed complex formation in systems where water is the second ligand. Typical values of $\log K_1 - \log K_2$ range from 0.5 to 0.8 for monodentate ligands and from 1 to 2 for bidentate chelates.[182] When a decrease in coordination number occurs on complexation, lesser differences appear, as shown for the ammonia and imidazole complexes of Zn(II) in Table II. Relatively large differences in $\log K_1 - \log K_2$ noted in section IV for Cu(II) complexes of histidine derivatives that chelate only in a histamine-like way indicate that these ligands avoid the binary com-

TABLE VII. Δ Log K for Mixed Divalent Transition Metal Ion Complexes[a]

Ligand A	Ligand B	Co	Ni	Cu	Zn	Ref
Imidazole[b]	Gly-Gly		−0.1	0.35		c
	NTA	0,1	0.0	−0,15	−0.2	d
	EDTA	0,8	0.8	1,4	0.7	d
4-Aminomethyl-imidazole	Catecholate			0.35		e
	AMP			0.35		f
Histamine	en	0.88	1.20	1,43	0,19	g
	Serinate	0.48	0,82	0.58	−0.17	g
	Catecholate			0.48		h
	NTA	1,5	2.0	3.4	2.0	i
Histidinate⁻	Threoninate			0.58		j
	Histidine⁰			0.55		j
	His methyl ester			1.8		k
	NTA	3,0	3,7	5,6	2,7	l
				4,4		m
Histidine⁰	Threoninate			0,17		j
	NTA			0.8		m
	EDTA			2.7		l
His methyl ester	NTA			3,6		m
Bipyridyl	en	0.27	0.18	1.29	0.49	n
	Glycinate	0.17	0,21	0.35	0.39	n
	Catecholate	−0.76	−0,36	−0.43	0.01	n
	AMP			−0,53		f

[a] Given by or calculated from results in references and at 25° unless otherwise indicated. [b] Log K_1 values for imidazole used in calculation taken from Table II. [c] References 178 and 185. [d] J. Israeli and H. Saulnier, *Inorg. Chim. Acta*, **2**, 482 (1968). [e] Reference 190. [f] Reference 189. [g] At 37°: D. D. Perrin, I. G. Sayce, and V. S. Sharma, *J. Chem. Soc. A*, 1755 (1967); D. D. Perrin and V. S. Sharma, *ibid.*, 446 (1968); 2060 (1969). [h] P. R. Huber, R. Griesser, B. Prijs, and H. Sigel, *Eur. J. Biochem.*, **10**, 238 (1969). [i] A. L. Beauchamp, J. Israeli, and H. Saulnier, *Can. J. Chem.*, **47**, 1269 (1969). Log K_1 values for histamine taken from Table II. [j] Reference 131. [k] R. W. Hay and P. J. Morris, *J. Chem. Soc. A*, 1518 (1971). [l] J. Israeli and M. Cecchetti, *J. Inorg. Nucl. Chem.*, **30**, 2709 (1968). Log K_1 values for histidine taken from Table II. [m] D. Hopgood and R. J. Angelici, *J. Amer. Chem. Soc.*, **90**, 2508 (1968). Log K_1 value for protonated histidine from footnote *j* and for histidine methyl ester from Table II. [n] R. Griesser and H. Sigel, *Inorg. Chem.*, **10**, 2229 (1971).

plexes on the left side of eq 16 in favor of a mixed ternary complex with water as the second ligand, MA.

Values of Δ log *K* given or calculated from results in the literature for imidazole derivatives and another ligand are presented in Table VII. Unfortunately relatively few results are available, but examples of conclusions elaborated on bipyridyl and other compounds[182] may be spotted in Table VII. Aromatic amines such as bipyridyl favor oxygen over nitrogen donors in mixed complex formation, and this trend is also noted for histamine in the series diaminoethane (en), serine, and catechol where Δ log *K* becomes progressively less positive. Serine and threonine chelate as substituted glycines in the pH range considered in this section.[140,183] (See Addendum.)

The special favoring of mixed complex formation with aromatic amines is looked at in another way in Figure 10. The distribution of complex species for equimolar amounts of Cu(II) and each of two ligands are plotted as a function of pH. As the pH increases the concentration of complexes with two added ligand molecules increases. In the bottom half of Figure 10 the distribution of complex species is nearly statistical at pH 8 with the mixed ternary complex of serine and diaminoethane representing half of the total Cu(II). When histamine replaces diaminoethane the percentate of the ternary complex leaps to 94%.

A similar analysis of published constants indicates that the ternary $Cu(His^-)(Thr^-)$ complex is 88% of total Cu(II) in equimolar solutions in the pH region where 2:1 complexes prevail. The lesser amount of mixed complex than for $Cu(Ser^-)(histamine)^+$ may be ascribed to an electrostatic contribution favoring the latter, positively charged, mixed complex. The identical Δ log *K* values (Table VII) for the two systems suggest similar structures in solution for the ternary complexes. Three nitrogen and one oxygen donor in the Cu(II) coordination plane are envisaged for both complexes with a weakly coordinated apical carboxylate in the histidinate containing complex as in the crystal structure.[127] This structure accounts for the observation that the ternary $Cu(His^-)(Thr^-)$ complex absorbs at a shorter wavelength than either binary 2:1 complex, while a proposed ternary structure with both ligands bound in glycine-like modes[184] does not appear to do so. The apically coordinated imidazole group is held responsible for the significantly longer absorption maximum in $Cu(His^-)_2$ (section IV).

These results support the idea (section IV) that acceptable structures for the $Cu(His^-)_2$ complex in solution should contain the two ligands bound in different modes to satisfy strong tendencies for mixed complex formation. In addition, the especially high Δ log *K* value for histamine and diaminoethane, indicating according to eq 15 that the ternary MAB complex is definitely not favored, appears to exclude a structure for $Cu(His^-)_2$ with four nitrogen donors in one coordination plane.

Values of Δ log *K* for the mixed histidinate complexes provide additional support for the solution structure of the protonated complex $Cu(His^-)(HisH)^+$ discussed in section IV. The Δ log *K* value for this ternary complex is nearly identical with that of $Cu(His^-)(Thr^-)$ but more than one log unit less than that for $Cu(His^-)$(histidine methyl ester)$^+$. Thus neutral histidine appears to bind to $Cu(His^-)^+$ in a glycine-like mode as does threonine rather than in a histamine-like mode as does histidine methyl ester.

Since EDTA occupies more of a coordination sphere than nitrilotriacetate (NTA), its Δ log *K* values are greater. High values for the Cu(II) complexes are consistent with its lesser coordination number compared to the other metal ions in Table VII. An informative comparison is provided by the mixed nitrilotriacetate complexes in Table VII. Monodentate imidazole yields the lowest value of Δ log *K*, neutral histidine HisH is next, the bidentate pair histamine and histidine methyl ester of quite different

basicity yield nearly identical values of 3.5 ± 0.1, and two studies for histidinate give discordant results but clearly the highest value by at least one log unit. The ternary MAB complex is most favored by the monodentate imidazole which binds about as strongly to $M(NTA)^-$ as to $M(aq)^{2+}$. The greater value of Δ log K for histidinate over the bidentate pair supports the suggestion (section IV) that it is bound as a terdentate ligand in the MA complex.

Rate reductions due to glycylglycine complexes in two different reactions catalyzed by imidazole were utilized to determine stability constants for formation of mixed complex, which is catalytically inactive. The calculated Δ log K values appear in the first row of Table VII (a result reported for Cd(II) appears compromised by incomplete formation of $Cd(Gly\text{-}Gly)^+$ under the experimental conditions).[185] The different nature of the Gly-Gly complex for Cu(II) compared to Ni(II) accounts for its greater Δ log K value. At the pH conditions employed Gly-Gly is terdentate to Cu(II) with amino and deprotonated amide nitrogens and a carboxylate oxygen donor while only bidentate to Ni(II) *via* amino nitrogen and amide oxygen donors. No amide hydrogen ionization has occurred in the last complex which has several more coordination positions available than the single one in the Cu(II) complex. Features of the crystal structures reviewed earlier in this section support this interpretation.

Two different studies report additional examples of ternary complex formation with Zn(II). A low concentration of $ZnCN^+$ prevents a quantitative evaluation by titration methods, but it does seem safe to conclude that for the ternary $Zn(His^-)(CN^-)$ complex Δ log K is negative.[186] Chemical shift trends in pmr spectra taken in DMSO were interpreted as evidence for ternary complexes of Zn(II) and imidazole with the nucleosides guanosine and cytidine.[34]

Mixed complex formation may also have important consequences on the rates of metal ion catalyzed reactions. The effectiveness of equimolar Cu(II) complexes as catalysts in the hydrolysis of diisopropyl fluorophosphate decreases in the order bipyridyl > histidine > imidazole (2:1) > amino acids such as alanine.[187] Some examples of reactivity of mixed complexes in redox reactions appear in section VIII.

The tendency of aromatic amines to favor mixed complex formation with oxygen donor ligands has been ascribed to π-acceptor properties of the amines.[182,188] The frequently studied bipyridyl is a better π acceptor than imidazole and derivatives, and its tendency to form mixed complexes is substantially greater.[189,190] The significance, if any, of the necessarily cis arrangement of aromatic nitrogens in bipyridyl complexes seems uncertain. For bipyridyl with Cu(II) there is a substantial steric contribution favoring formation of mixed tetragonal complexes compared to the 2:1 bipyridyl complex as the last, nontetragonal complex provides an inappropriate reference state for tetragonal complexes.[191] The importance of π interactions as contributor to mixed complex formation with aromatic amines is supported by the change in the sign of the net circular dichroism in the d-d absorption region of L-alanine and (*R*)-1,2-diaminopropane complexes upon replacement of similar ligands across the Cu(II) coordination plane with histamine, bipyridyl, o-phenanthroline, and dipyridylamine.[140a,191]

The tendencies toward mixed complex formation reviewed in this section have far-reaching implications for likely binding sites of metal ions in proteins. By reason not only of its basicity, but also its tendency to form mixed complexes, the imidazole side chain on histidyl residues presents a likely binding site for transition metal ions. However, the tendency of aromatic amines to form mixed complexes, especially with ligands containing negatively charged oxygen donor atoms, suggests that binding sites will not contain imidazole groups exclusively, but will also show other donor atoms including carboxylate oxygens. Unfortunately few examples exist to assess the tendency of an ionized peptide nitrogen to enter into mixed complex formation, but it may be similar in its proclivity to a carboxylate oxygen. We also anticipate that phosphate groups of substrates might bond favorably in a ternary complex to a histidyl bound metal ion on a protein. Some examples of these ideas are cited in section VI.

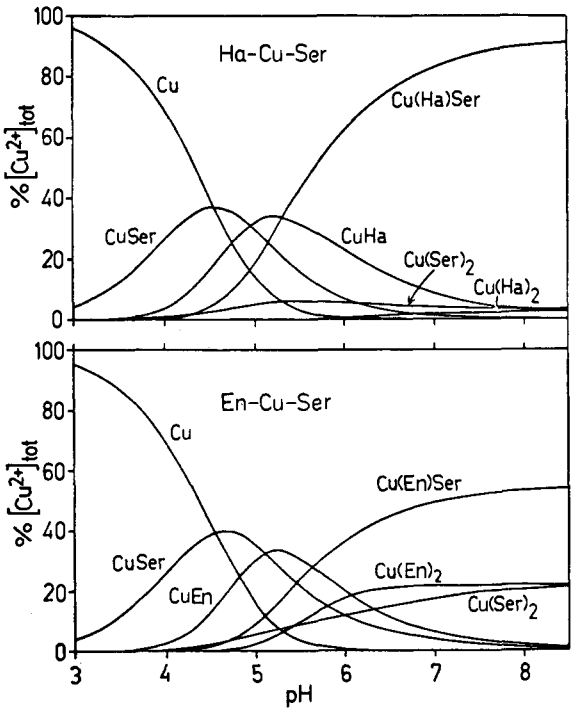


Figure 10. Relative concentrations of complex species as per cent of total Cu(II) in aqueous solution containing 10^{-3} *M* total Cu(II) and each of two ligands over a range of pH at 37°. Upper figure: histamine (Ha) and serine (Ser) ligands. Lower figure: diaminoethane (en) and serine ligands. The possibility of hydrolysis at high pH was omitted from the calculations. Figure is from ref 182 and is based on equilibrium constants presented by D. D. Perrin, I. G. Sayce, and V. S. Sharma, *J. Chem. Soc. A*, 1755 (1967).

VI. Complexes of Peptides and Proteins

The histidyl residues of proteins play a prominent role in the binding of metal ions, as will be discussed in the succeeding subsections. First we describe ways in which histidyl residues affect metal ion binding in peptides. This discussion is followed by one of the binding of externally added metal ions to rather specific histidyl sites in proteins that do not usually require those metal ions for function. Finally this section concludes with a discussion of metalloproteins, where the metal ions are found in the native protein, are usually strongly bound, and are often necessary for structure and function. As indicated, a variety of physical and chemical methods have been utilized to ascertain involvement of histidyl residues in metal ion binding.

A. Peptides

Carnosine (β-alanyl-L-histidine) is found in vertebrate muscles and its function is uncertain. Because of the β-

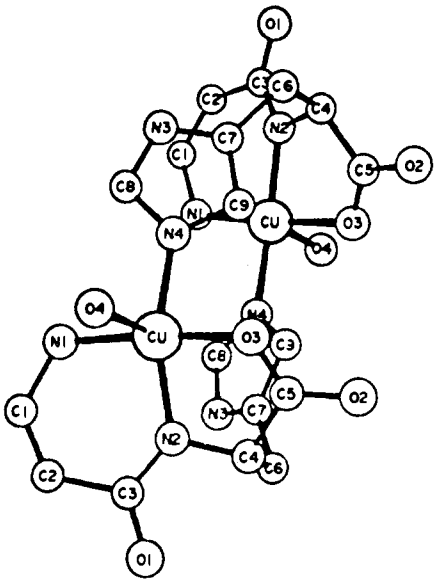


Figure 11. Dimeric complex of Cu(II) and carnosine (β-alanyl-L-histidine). The N-1 and N-3 nitrogens of the imidazole ring referred to in the text correspond to N3 and N4, respectively, in the figure. Reproduced from ref 25.

alanyl residue, the dipeptide is not a fragment of proteins, which contain only α-amino acid residues. The additional methylene group of the β-alanyl residue results in a chelate structure different from that of glycylhistidine. In a solid the structure of the carnosine complex of Cu(II) is a dimer and the result of a crystal structure determination is shown in Figure 11.[25] Each Cu(II) is bonded to an amino nitrogen, a deprotonated amide nitrogen, and a carboxylate oxygen, the ligand chelating as would β-alanylglycine. The dimer is joined by a basic pyridine 3-nitrogen of the imidazole ring of each ligand complexing to the fourth coordination position of the other Cu(II). Coordination about Cu(II) may be described as square pyramidal with Cu(II) departing from the mean plane of three nitrogen and one carboxylate oxygen donors toward an apical water molecule.

Several lines of evidence indicate that the dimer structure of the carnosine-Cu(II) complex first found in the crystal also exists in solution. Titration curves of equimolar solutions of Cu(II) and either carnosine or anserine (β-alanyl-L-1-methylhistidine) are virtually superimposable and show by pH 7 formation of a species of zero net charge.[192,193] Amide hydrogen ionization occurs from pH 5 to 6. It is not possible to describe a sterically feasible monomeric structure without invoking Cu(II) coordination to a N-1-methylated pyrrole-type nitrogen in the imidazole ring, and the prohibition against doing so was explained in section II. Because the imidazole ring N-3 is bound in the dimer, methylation at N-1 does not affect the structure shown in Figure 11. In addition, a second mole of ligand in a 2:1 solution containing either carnosine or anserine and Cu(II) titrates as if unbound.[138,193] Because all coordination positions about Cu(II) are occupied in the dimer, it can account for freely titrating additional ligand. The CD of an equimolar solution of carnosine and Cu(II) is unlike that of other histidine-containing dipeptides and is unaffected by the presence of additional ligand.[134] The dimer becomes the dominant species in neutral equimolar solutions of carnosine and Cu(II) when the total carnosine concentration is $>5 \times 10^{-4}$ *M*. The dimer went undetected by electron spin resonance in aqueous solutions at room temperature.[194]

A careful determination of selective broadening in the proton magnetic resonance spectra of carnosine by Cu(II) over a wide pH range has been subjected to an exhaustive analysis by Ihnat and Bersohn[195] (I and B hereafter). They assume a dipolar (r^{-6}) interaction between the paramagnetic ion and hydrogens of the ligand that are present in about 2000-fold excess to calculate probable complex structures. Their interpretation of complex structures differs from the dimer found at stoichiometric ratios in both the solid and solution at pH >6. We offer an alternative interpretation to that of I and B for their conditions of excess ligand at pH >8. It is only the abundance of their results that permits such a detailed critique of the study of I and B.

TABLE VIII. Pmr Relative Band Width Increments for Cu(II) and Carnosine[a]

Hydrogen	Observed	Models 2 and 7	Models 2 and 3
Im H-2	93	100 (2)	100 (2)
Im H-4	100	100 (2)	100 (2)
α-H	3.6	38 (7)	3.1 (2)
$NH_3^+CH_2$	88	88 (7)[b]	88 (3)[b]
CH_2CO	4.6	38 (7)	10 (3)
CH_2(His)	4.2	27 (7)	3.1 (2)

[a] At pD 11.4 from ref 195. [b] Calculated $NH_3^+CH_2$ bandwidths scaled to observed value of 88 for models 7 and 3.

As the basicity of a solution containing carnosine and Cu(II) is increased from pD 2, the bandwidth increment of the CH band is first to increase, followed by the pair of imidazole protons which reach a maximum band width at pD 7.4, then begin to sharpen as the basicity is increased further, giving a plateau in the pD 11.4 region before becoming very sharp at pD 13. The bandwidth of CH_2CO twice reaches maxima at pD 7.8 and 10.9. The broadening of CH at the lowest acidities followed by imidazole protons in neutral solutions is consistent with monodentate binding of Cu(II) first to carboxylate, then to imidazole sites as the basicity increases. This monodentate binding in acid and neutral solutions agrees with the interpretation of I and B up to pH 8.

The most detailed comparison of observed and calculated bandwidth increments was performed at pD 11.4 and is presented in Table VIII. I and B considered several models for Cu(II) interaction at carnosine and favored a mixture of Cu(II) binding at Im N-3 (model 2) and chelation by amino and ionized amide nitrogens and a carboxylate oxygen (model 7). Their estimated relative bandwidths with this mixture are listed in the third column of Table VIII. Agreement with the observed results in the second column is poor for three of the six carbon-bound hydrogens where the mixture of models 2 and 7 predicts bandwidths almost ten times greater than observed. Much better agreement with the observed results in the second column is obtained by considering a mixture of Cu(II) binding at Im N-3 (model 2) and the amino nitrogen (model 3). The results that would be predicted by I and B for a mixture of models 2 and 3 are presented in the last column of Table VIII. The agreement of this last combination (not mentioned by I and B) with the observed results is within the limitations of the calculated bandwidths derived from assumed models. A more complete analysis of the implied binding of Cu(II) at N-3 in preference to N-1 of the imidazole ring should consider the role of possible contact contributions to the broadening mentioned in section IV. As it has for proton binding sites,[196] carbon-13 nuclear magnetic resonance should prove useful for determination of metal ion binding sites in histidine derivatives.

The conclusion from Table VIII that at pD 11.4 Cu(II) binds to excess carnosine *via* imidazole and amino nitrogens has been suggested before[192] and is supported by

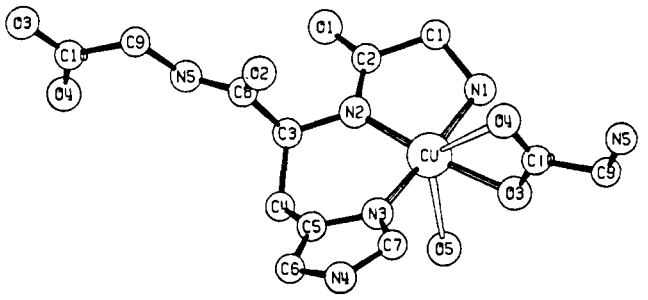


Figure 12. Crystal structure of Cu(II) complex of glycyl-L-histidylglycine showing amino, deprotonated peptide nitrogen, and imidazole nitrogen donor atoms of ligand. Coordination about Cu(II) is completed by a carboxylate oxygen from a second ligand (O4) and a water molecule (O5), both of which interact weakly. Reproduced from ref 203.

experiments on the rates of substitution of water by carnosine on Cu(II). In neutral solutions containing approximately equimolar amounts of carnosine and Cu(II), relatively slow substitution rates are observed, indicating that the substitution reaction is under steric control; the rate of water reentry in place of the first ligand donor atom in the primary coordination sphere is greater than the rate of coordination of the second ligand donor atom on a chelatable ligand.[197] In complexes where chelation is under steric control, ring formation will not occur before the metal ion passes to another ligand molecule. Chelation at an amide nitrogen demands its deprotonation. The very slow deprotonation of the amide hydrogen assures that all peptide complexes chelated at the amide nitrogen are formed under steric control.[198] Thus in a selective broadening experiment with peptides, Cu(II) binds only in monodentate or weakly bidentate modes as it passes rapidly from one excess ligand molecule to another.[199] Carnosine bound by an ionized amide nitrogen indicated in model 7 of I and B is specifically ruled out by the slowness of the deprotonation step.

Finally the sharpening of all bands in the pmr spectra of blue solutions of Cu(II) and excess carnosine at pD 13 was not explained by I and B. The sharpening is easily accommodated by chelation rendering the Cu(II) nonexchangeable on the rapid pmr time scale. At high pH carnosine molecules with ionized amide hydrogens exist in sufficient amount to chelate Cu(II) and form complexes similar to the dimers found at stoichiometric concentrations in solid and solutions resulting in an inhibition of exchange of Cu(II) among excess ligands. Strong chelation slows exchange and renders the metal ion ineffective in interacting with excess ligand so that little broadening is observed.

The carnosine complexes formed by metal ions other than Cu(II) are uncertain. Titrations have been performed on solutions containing carnosine and several metal ions by two groups of investigators who arrive at conflicting conclusions and even report different results. In the first study stability constants were calculated assuming initial association with the imidazole ring of excess ligand, and the results obtained are in line with those of Table II for association of the same metal ions with imidazole.[192] In the second study stability constants calculated from titration results on equimolar solutions yield similar high values for several metal ions which are interpreted in terms of a structure with chelation at the imidazole ring and the carboxylate group.[21] The values quoted appear too high for the structure proposed when compared with stability constants for imidazole (Table II), acetylhistidine,[192] or imidazole-4(5)-propionate.[118] The results for the Ni(II) complex are described as unusual,[21] but the titration curve shown differs from one published earlier[193] and does not seem to be reproducible.[138] Finally, all the structures drawn in ref 21 are incorrect as they invoke metal ion coordination at pyrrole-type nitrogens of the imidazole ring, which is unlikely (section II), and in one case also at an un-ionized amide, which is also improbable, as reviewed below.

TABLE IX. Metal Ion Promoted Amide Hydrogen Acidity Constants[a]

	Cu(II)[b]			Ni(II)[c]		
	pK_1	pK_2	pK_3	pK_1	pK_2	pK_3
AcGly-Gly-His	6.5	7.4	9.3	8.7	9.0	9.2
$Ac(Gly)_2$-His-Gly	6.0	6.5	9.0	8.4	8.5	8.6
Gly-Gly-His	4.9	5.0		6.3	6.4	
Gly-Gly-Gly	5.1	6.9		8.3	8.5	
Gly-Gly-Gly-Gly	5.9	7.0	9.3	8.1	8.2	8.3
Pentaglycine	6.1	7.0	8.2			

[a] At 25° and 0.16 ionic strength. [b] G. F. Bryce, R. W. Roeske, and F. R. N. Gurd, *J. Biol. Chem.*, **240,** 3837 (1965); ref 216; C. R. Hartzell and F. R. N. Gurd, *J. Biol. Chem.*, **244,** 147 (1969). [c] References 234 and 206.

For glycylhistidine the ligand donor atoms deduced from titration results for the equimolar Cu(II) complex in neutral solutions[192] are the same as those found in a crystal structure determination.[200] The dipeptide serves as a terdentate ligand with amino, ionized amide, and imidazole (pyridine) nitrogen donor atoms that form one five and one six-membered chelate ring as illustrated for a similar complex in Figure 12. In the crystal the coordination square pyramid about Cu(II) is completed by a carboxylate oxygen from a second complex and an apical water molecule, toward which the Cu(II) is displaced from the coordination plane. The neutral complexes are not polymeric in solution so that the carboxylate group is unbound. Titration results and absorption spectra indicate that hexacoordinate Ni(II)[192] and tetragonal Pd(II)[155] also form neutral complexes with three nitrogen donor atoms to the metal ion in neutral solutions. Tetrameric complexes of the three metal ions formed upon increasing the pH to >9 are discussed in section IX. Solutions containing equimolar amounts of Co(II) and Gly-His give under nitrogen a neutral complex by pH 9 in which amide hydrogen ionization has evidently occurred and a quadridentate ligand was suggested.[201] Oxygenation of solutions containing Co(II) and Gly-L-His yield Co(III) complexes in which amide deprotonation has also evidently taken place. Several products have been obtained and their structures appear less certain than those of the other transition metal ion complexes.[201,202]

Complexes of Cu(II) with glycylhistidylglycine have been studied in solution and in the crystalline phase. As for glycylhistidine, the basic structural unit contains a Cu(II) ion ligated by a terminal amino group, an ionized peptide nitrogen, and an imidazole nitrogen as shown in Figure 12. Coordination about Cu(II) is completed by an oxygen from the carboxylate group of another ligand and a water molecule. In a second structure two copper ions are bridged by carboxylate groups from two other peptide molecules. It is also concluded from titration data that similar species exist in solution, including clusters containing five and six dimeric Cu units.[203]

Ionizations of amide hydrogens that normally occur from pH 13 to 15 in peptides are markedly promoted in the presence of Cu(II) or Ni(II) when a histidyl residue or α-amino group is available to serve as an anchor for the metal ion. As shown by the pK_a values listed in Table IX for a selection of peptides in the presence of equimolar amounts of Cu(II) or Ni(II), promotion of peptide ionizations is often so great that they occur at higher acidity

than physiological pH. The first peptide tabulated, acetylglycylglycylhistidine, and the second with an additional glycyl residue may be taken as models for an internal histidyl residue in a protein. Cu(II) and Ni(II) promoted peptide ionizations occur to the amino group side of histidyl residues in polypeptide chains. Deacetylation to give the third tabulated peptide provides a second anchor for either metal ion and results in about a two log unit lowering of the pK_a for the second amide hydrogen to ionize (pK_2). This ionization also occurs about two pK_a units lower than in amino terminal peptides that do not contain a histidyl residue, as shown by the last three entries in Table IX. Thus a histidyl residue in the third position from an amino terminus is especially effective in abetting Cu(II) and Ni(II) promoted peptide ionization.

After completion of peptide ionizations, the deprotonated nitrogen coordinates to the transition metal ion so that all the ligands of Table IX form complexes with three chelate rings. Except for Gly-Gly-Gly, which possesses only three nitrogens, the peptide complexes of Table IX contain four nitrogen donors coordinated in a tetragonal plane. The tetragonal triglycine complex possesses three nitrogen and one carboxylate oxygen donors. These structures give rise to the biuret colors which are red-violet with Cu(II) and yellow with Ni(II). Amino, ionized peptide, and imidazole nitrogens are not distinguishable in room temperature electron spin resonance spectra of Cu(II) complexes of histidyl containing peptides.[204]

Only deprotonated amide nitrogens coordinate to metal ions: the numerous structures drawn in the literature showing coordinated but un-ionized peptide nitrogens are incorrect. The most basic site in an amide linkage is the carbonyl oxygen so that either a proton[204a] or a metal ion[38,205] associates at that atom. Upon deprotonation of an amide nitrogen it becomes the most basic site, and only then does protonation or metal ion coordination take place at the nitrogen atom. When either protonation or metal ion coordination has occurred at an ionized amide nitrogen, the oxygen again becomes the most basic site as illustrated by structures of cobalt(III) complexes of glycylglycine.[205a]

Several of the peptide complexes in Table IX exhibit closely spaced pK_a values indicative of cooperative ionizations. Such deprotonations occur over a narrow, strongly buffered pH region, and the individual pK values furnish a quantitative expression of the extent of cooperativity. Halfway through titration of the amide hydrogens in an equimolar solution of Gly-Gly-His and Cu(II) or Ni(II), half of the complexes contain two amide hydrogens and half none, rather than all of the complexes containing one ionized amide nitrogen. For Cu(II) cooperativity is observed only for Gly-Gly-His where the amino anchor at one end of the peptide and the imidazole nitrogen at the other form the termini for three chelate rings only upon deprotonation of the two intervening amide nitrogens. In Ni(II) complexes cooperativity is associated with a change from octahedral, paramagnetic, blue complexes to tetragonal, diamagnetic, yellow complexes.[206] As a result of this cooperativity, tetraglycine forms a stronger complex with Ni(II) than with Cu(II) at pH >8.5.[206] The yellow tetragonal Ni(II) complexes display absorption maxima from 410 to 490 nm which include magnetic dipole allowed d-d transitions.[207,208] No such transitions occur in the visible region of the spectra of octahedral Ni(II) complexes and thus they exhibit only weak circular dichroism (CD) with optically active ligands. The diagnostic value of the significant CD displayed by the yellow, tetragonal complexes of Ni(II) with optically active peptides is illustrated in the next subsection.

Owing to the presence of large side chains and other attributes of proteins such as conformation, we do not anticipate an exact correspondence between transition metal ion interactions with proteins and the peptides of Table IX. Steric inhibition of peptide hydrogen ionization in transition metal ion complexes has been proposed.[205] We expect that the secondary structure of a protein will severely limit the number of ionized peptide nitrogens to the amino terminal side of an internal histidyl residue that can chelate to a metal ion coordinated at the imidazole side chain. Therefore, in extrapolating the values in Table IX to the behavior of Cu(II) and Ni(II) interaction in proteins, we anticipate that the metal ions will simply coordinate to imidazole side chains from pH 5 to 6, and that coordination at the amino terminus and chelation at succeeding ionized peptide nitrogens will take place progressively as the pH is raised. Neutral solutions then provide a transition region for chelation of Cu(II) and Ni(II) at the amino terminus, which should be a general interaction of these two metal ions with proteins.[209] Except when the histidyl residue is the second or third from the amino terminus, chelation of the metal ions at adjacent ionized peptide nitrogens is expected to be rare. This discussion of small peptide complexes provides a reference frame for interactions of Cu(II) and Ni(II) with albumins and ribonuclease reviewed below.

Poly-L-histidine avidly binds one Cu(II) for every four histidyl residues.[209a] The binding is complete by pH 5, and titration indicates that an average of one ionized peptide nitrogen is involved in binding each Cu(II). CD and esr spectra are presented and a possible structure of the complex is advanced. The oxidase activity of the peptide-Cu(II) complex is mentioned in section VIII.

B. Metal Ion–Protein Interactions

Numerous studies have reported binding of transition metal ions to proteins, and on the basis of the similarity of formation constants to those with imidazole, complexation at the histidyl residue of proteins has often been inferred. We limit this review to recent studies which utilize modern concepts and techniques to provide some specific evidence for histidyl side chain involvement in binding of transition metal ions.

In humans a significant portion of the exchangeable Cu(II) in blood is transported by the albumin fraction of blood serum. Albumins appear to consist of single polypeptide chains with molecular weights of about 65,000. Reports that bovine serum albumin (BSA) binds relatively strongly one mole of Cu(II) per mole of protein at a site other than the lone sulfhydryl group led to the suggestion that the first equivalent of Cu(II) is bound at the amino terminal aspartyl residue.[210] Resistance of this residue to dinitrophenylation in the presence of an equivalent of Cu(II) supported interaction at the amino terminus.[211] Cu(II) binding to BSA is stronger than to amino acid aspartate and is inhibited by Ni(II) but not by Zn(II).[210] Direct experiments with Ni(II) showed that an equimolar solution with BSA yields the same yellow color and absorption maximum from 410 to 450 nm observed in small peptide complexes. As a result it was suggested that the first equivalents of Cu(II) and Ni(II) react not merely with the amino terminal residue of albumins, but with the amino nitrogen and with chelation at succeeding deprotonated peptide nitrogens.[209] A yellow absorption band was also observed upon addition of Ni(II) to human serum albumin (HSA) which possesses the amino terminal sequence Asp-Ala-His-, and it was also suggested that the histidyl residue in the third position made interaction at the amino group and two deprotonated peptide nitrogens

more likely.[209] These ideas were subsequently substantiated by studies of the small peptide complexes reviewed in Table IX.

Further studies concentrated on the interaction of the first equivalent of Cu(II) with BSA. Proton displacement upon addition of Cu(II) to BSA and the similarity of the visible absorption spectrum to that observed with Cu(II) peptides after peptide ionization suggested that two peptide nitrogens are coordinated in addition to the α-amino nitrogen.[212] Involvement of a β-carboxylate oxygen of the amino terminal residue was also proposed. Participation of the histidyl residue in Cu(II) binding to BSA, in conformity with the earlier suggestion for Cu(II) and Ni(II) interactions with HSA,[209] was advanced on the basis of studies with the amino terminal 24 residue peptide fragment of BSA[213] which contains the amino terminal sequence Asp-Thr-His-Lys-.[214] This tetrapeptide, the 24-residue peptide, and BSA on binding of 1 equiv of Cu(II) all yield similar proton displacements plus visible absorption and circular dichroism spectra.[215] Binding of 1 equiv of Cu(II) to the 24 residue peptide fragment protects the α-amino group and the histidyl residue in position 3 from carboxymethylation.[215] Thus the inference is strong that the first equivalent of Cu(II) added to BSA binds to an α-amino nitrogen, two intervening ionized peptide nitrogens, and an imidazole nitrogen from the histidyl residue in position 3. A second equivalent of Cu(II) appears to complex about equally with two interior histidyl residues of the 24 residue peptide fragment.[215]

Visible circular dichroism results for interaction of Cu(II) and Ni(II) with peptides and BSA are summarized schematically in Figure 13. Complexes with ionized peptide nitrogens containing L-amino acids typically give negative CD with Cu(II)[140] and Ni(II).[207] An L-histidyl residue with chelated adjacent ionized peptide nitrogens yields a long wavelength negative CD and a short wavelength positive CD[216] as illustrated in the second row of Figure 13. The CD result for interaction of Ni(II) at the sulfhydryl locus of glutathione differs in wavelength and pattern from that for its complexation with histidyl residues. The first equivalent of transition metal ion added to BSA suggests interaction at a histidyl residue, and additional equivalents up to four interact at the sulfhydryl locus.[217] These diagnostic CD results support earlier suggestions that the first equivalent of Cu(II) or Ni(II) does not react at the sulfhydryl group of BSA but at the amino terminal histidyl containing tripeptide. Not illustrated in Figure 13 is the CD developed near 310 nm when Cu(II) chelates with ionized peptide nitrogens of L-amino acid residues. This ultraviolet CD peak with Cu(II) and the yellow color of Ni(II) complexes signal involvement of ionized peptide nitrogens with each metal ion, respectively.

Similar amino terminal tripeptides with a histidyl residue in the third position are found in human, bovine, and rat albumins. All three albumins bind strongly 1 equiv of Cu(II) in neutral solutions. Cu(II) is not bound as strongly to dog albumin, where higher pH is required to effect the biuret color indicative of Cu(II) binding to ionized peptide nitrogens.[218] In dog albumin the histidyl residue is replaced by tyrosine,[219] indicating again that the histidyl residue in the third position is crucial for a highly preferred amino terminal Cu(II) binding site in neutral solutions of albumins. The lack of a His-3 in dog albumin is also evident in CD experiments.[219a] Upon addition of 1 equiv of Cu(II), HSA gives a CD spectrum similar to that of BSA shown in Figure 13. Dog albumin, however, at pH 10 yields a CD minimum near 530 nm, characteristic of Cu(II) chelation at the amino terminus and three succeeding ionized amide nitrogens, without involvement of a histidyl residue.[140] The amino acid histidine withdraws Cu(II) from the quadridentate amino terminus site of HSA to form a histidine-Cu(II) complex and a small amount of a ternary complex of unknown structure.[220]

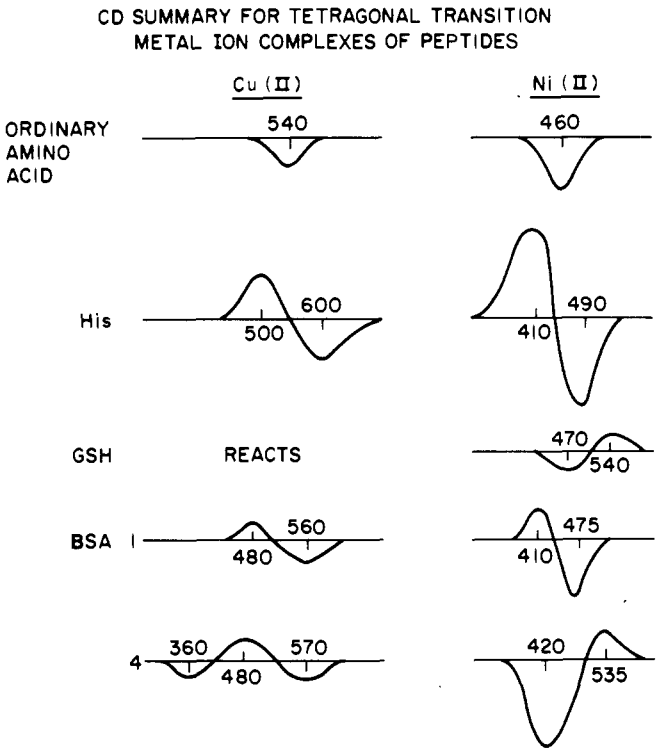


Figure 13. Schematic circular dichroism curves in nm for addition of Cu(II) and tetragonal Ni(II) to peptides containing L-amino acid residues. In the first three rows appear curves for peptides containing ordinary amino acid residues, histidyl residues, and glutathione (γ-L-glutamyl-L-cysteinylglycine) as a model for the sulfhydryl side chain. In the last two rows 1 and 4 equiv of the transition metal ions are added to bovine serum albumin, BSA.

It is known that aquocobal(II)amin binds to bovine serum albumin. The binding is reversed by cyanide. From spectral data, the pH dependence of the binding, and inhibition of binding by Cd(II) it has been concluded that imidazole rings of histidine residues are responsible for the binding, presumably interacting by displacing water from the sixth coordination site on cobalt.[221,222] The binding is believed to be rather nonspecific and distinct from the specific binding of cobalamins to enzymes involved when the cobalamin is a substrate or reacting coenzyme. This type of nonspecific binding may, however, be involved in transport of B_{12} in serum. Competition experiments in the presence of an equivalent of Cu(II) or Ni(II) may establish whether His-3 of albumin is involved in binding of aquocobalamin.

The activity of bovine pancreatic ribonuclease is inhibited by Cu(II), and investigations have been conducted into the sites of metal ion binding to the enzyme. Perhaps, because it does not bind Cu(II) especially strongly or specifically, as shown by the discovery of seven sites by X-ray diffraction of the crystalline enzyme prepared at pH 5.5,[223] not all studies of even the same investigators have reached identical conclusions. Some of the inconsistencies are resolved if attention is paid to the pH of the measurement and the principles developed near the end of section VI.A concerning competition between histidyl residues and the amino terminus as potential binding sites. Potentiometric and spectrophotometric results were interpreted in terms of four binding sites of similar strength, and the four histidyl residues were selected as

the likely sites.[224] This conclusion was later modified by a gel filtration study from pH 5.5 to 7 to four weak binding sites identified as before and one strong binding site suggested to be the amino terminus.[225] Increasing amounts of acetate at pH 7 favor binding at the four weaker sites at the expense of the one stronger site. We may offer supporting arguments for the site identification at pH 7, as binding of Cu(II) to the aromatic imidazole groups should also favor binding of negatively charged oxygen donors such as acetate in mixed complexes according to the conclusions of section V. In addition, binding at the amino terminus with peptide hydrogen ionization should be inhibited by acetate. Significant amino terminal ionized peptide nitrogen interactions for Cu(II) at pH 8.5 and Ni(II) at pH 10.5 are indicated by CD spectra.[217] For both metal ions only negative visible CD appear, as in the first row of Figure 13, suggesting little interaction of ionized peptide nitrogen donors and an adjacent histidyl residue. Appearance of CD near 320 nm for the Cu(II) and a yellow color for the Ni(II) complex indicate involvement of ionized peptide nitrogens for both metal ions. Strong support for the α-amino locus as the strong Cu(II) binding site at pH 7 in ribonuclease comes from comparison of the normal enzyme with deaminated derivative in which the α-amino group has been converted to an OH group.[226] The comparative gel filtration and spectrophotometric studies were also performed at pH 5.5 where the α-amino group in the normal enzyme is identified as one of the four weaker sites, leaving a histidyl residue as the strongest binding site at the low pH. Recent work identifies the strong amino terminus Cu(II) binding site at pH 7 in ribonuclease with a CD minimum at 710 nm.[226a] However, strong Cu(II) binding at amino terminal sites is characterized by a CD minimum near 540 nm with little CD intensity at 710 nm (Figure 13).

More specific information concerning the sites of Cu(II) binding to histidyl residues of ribonuclease is made available by chemical modification and nuclear magnetic resonance investigations. At pH 5.5 Cu(II) inhibits alkylation by iodoacetate of His-12 and His-119, suggesting that these two residues, both at the active site, are also involved in Cu(II) binding.[227] An equilibrium dialysis study of Cu(II) binding in the presence of β-alanine at pH 6.1 and 7.0 reveals one weak and one strong binding site, the latter of which correlates with inhibition of enzyme activity. This strong binding site disappears in the enzyme carboxymethylated at His-119.[227a] A marked enhancement of the water proton relaxation rate of carboxymethylhistidine-12-RNase in the presence of Cu(II) was interpreted to indicate that His-12 is the strongest binding site at pH 5.[228] In a similar way His-105 and His-119 were identified as weaker binding sites. Selective broadening by Cu(II) of the imidazole H-2 resonances in proton magnetic resonance spectra at pH 6 is greatest for His-105 and His-12, less for His-119, and least for His-48.[229]

Apparently discordant results on Cu(II) binding to the amino terminal 20 residue S-peptide from ribonuclease may be resolved by taking into account pH and the properties of the system to which the experimental measurements respond. Oxidative degradation and cleavage of the peptide at Thr-3 by chloroiridate in the presence of an equivalent of Cu(II) at pH 8 led to the conclusion that the Cu(II) is bound *via* the four amino terminal residues.[230] However, it does not seem necessary, according to the mechanism proposed,[231] to involve four amino terminal residues in bonding to Cu(II) to obtain degradation at Thr-3. At pH 8 selective broadening by Cu(II) implicates binding at His-12, the only histidyl residue in ribonuclease S-peptide.[229] Although not discussed by the author, this work provides strong evidence for Cu(II) binding at the amino terminus of S-peptide at pH >9 where a sharpening of the pmr spectrum was noted along with appearance of a pink (biuret) color. Since the peptide is present in at least tenfold excess, the sharpening of the spectra is due to removal of rapidly exchanging Cu(II) as, at the higher pH, a greater fraction is bound in a chelate complex and undergoes only slow exchange, similar to the interpretation offered earlier for carnosine. The chloroiridate oxidation experiments are responsive to Cu(II) bound at the three amino terminal residues, and the selective broadening experiments point to rapidly exchangeable Cu(II) typical of binding at imidazole side chains and not to chelation at the amino terminus. At pH 8 a transition is occurring (Table IX) from predominant Cu(II) binding at imidazole side chains at lower pH to chelation at the amino terminus at higher pH. Selective broadening responds to the low pH phase of this transition and the chloroiridate oxidation to the high pH phase.

Copper ions have an interesting effect on radiolytic destruction of proteins. The case of ribonuclease has been investigated most thoroughly. The salient features of the effect of Cu(II) are (1) increased rate of radiolytic damage and (2) increased destruction of specific amino acids. It has been suggested that the Cu(II) functions as an electron donor toward electron deficiencies caused by electron abstraction from the protein by radiolytically generated OH. The resulting Cu(III) species is considered responsible for structural damage to nearby amino acids, especially the coordinating groups. In ribonuclease the potential ligand groups histidine and proline are selectively destroyed by radiolysis in the presence of Cu(II).[232]

Sperm whale metmyoglobin has been shown by X-ray diffraction of a crystal to bind specifically 1 equiv of Cu(II) at His-A10.[233] Zn(II) binds at a different nearby site that probably involves His-GH1. In solution several Cu(II) react with metmyoglobin and apomyoglobin, and interactions at both histidyl residues and the amino terminus with peptide ionizations have been suggested.[212,234,235] Zn(II) competes with Cu(II) for some of the sites[236] but is not expected to promote peptide hydrogen ionization. Alkylation of the histidyl side chains reduces the ability of metmyoglobin to bind Cu(II).[235]

Insulin crystallizes in the presence of zinc ion to form a crystal with six insulin units and two zinc ions. Electron spin resonance spectra of a single isomorphous Cu(II) crystal indicate that each metal ion lies on the trigonal axis of the crystal and is bound to three nitrogen atoms.[237] Titration results on zinc insulin suggest that all three nitrogen atoms are provided by histidyl side chains.[238] These conclusions from esr of the Cu(II) crystal and titration of zinc insulin have been confirmed by the crystal structure of hexameric insulin with two zinc ions.[239] Zinc is bound only to histidyl residue B10 while histidyl residue B5 is exposed. Curiously, one histidyl residue is iodinated[240] and none carboxymethylated[241] in the presence of zinc ion, but two histidyl residues undergo each reaction in the zinc free monomer. The course of replacement of Zn(II) in insulin by vanadyl ion, VO^{2+}, has been followed by electron spin resonance spectroscopy.[242]

C. Metalloproteins

Carboxypeptidase A stands as an example of a metalloenzyme where chemical methods failed to identify correctly residues involved in binding of the single zinc ion

at the active site. An active site histidyl residue was not evident from carboxymethylation and photooxidation experiments.[243] Moreover, Zn(II) binding at a sulfhydryl group was indicated by reaction of only the apoenzyme with sulfhydryl reagents.[243,244] In addition, a combination of nitrogen and sulfhydryl donors was invoked to accommodate the stabilities of several metallocarboxypeptidases when other metal ions replace Zn(II).[245,246] Involvement of the terminal α-amino group in Zn(II) binding was also suggested.[243,246] On the other hand, the results of an X-ray diffraction study of the crystalline enzyme[247] in conjunction with the amino acid sequence[248] indicate that the zinc ion is bound to two histidyl and one glutamic acid residues about 25 Å from the amino terminus and that the sulfurs from the only two cysteinyl residues form a disulfide cross link. It is not entirely clear why chemical methods which have proved reliable with other proteins implicate a pseudo sulfhydryl site in carboxypeptidase A.

Other metal ions may be substituted for zinc ion in carboxypeptidase, sometimes resulting in increases of the peptidase and esterase activities observed for the native zinc enzyme.[246,249] The order of peptidase activity for metallocarboxypeptidases is Co(II) > Zn(II) > Ni(II) > Mn(II). For esterase activity the order is Mn(II) > Cd(II) > Co(II) > Zn(II) > Hg(II) > Ni(II) > Pb(II). Metal ions appearing in the latter list and not in the former yield a protein that is inactive as a peptidase. The Cu(II) substituted protein is inactive in both reactions though both kinds of substrates are bound.[250]

The two histidyl residues at the active site of carboxypeptidase serve mainly to hold the zinc ion, and a more profound role has not been suggested in the mechanism of hydrolysis of the carboxyl terminal amino acid residue of peptides.[247] However, the principles described in section V suggest that since complexes with imidazole ligands favor oxygen donors, binding of the carbonyl oxygen of the susceptible peptide bond of the substrate may be facilitated. The suggestion from the X-ray study that tyrosine-248 donates a hydrogen bond to the amide nitrogen of the susceptible peptide bond in the ground state of substrate binding disagrees with the principles developed in subsection VI.A. An amide nitrogen cannot be an acceptor of a proton or a metal ion, and this restriction holds with even more force when the amide carbonyl oxygen is interacting with a proton or a metal ion. Unless there is enormous strain associated with substrate binding, the earliest stage in the hydrolysis mechanism during which the peptide nitrogen may become a proton or hydrogen bond acceptor is concerted with nucleophilic attack at the peptide carbon which results in both the carbon and nitrogen of the peptide bond taking on tetrahedral character.

Thermolysin, an endopeptidase, contains one zinc ion at the active site and four calcium ions per molecule of enzyme. An X-ray structure analysis in conjunction with the complete amino acid sequence determination reveals that Zn(II) is bound by two histidyl and one glutamate side chains.[251,252] These donor groups are identical with those of carboxypeptidase A, and the relation of groups in the active site region of the two enzymes has been compared.

Concanavalin A binds Ca(II) and Mn(II) only 4.3 Å apart in a double site according to an X-ray diffraction study.[253] The Mn(II) is bound by one histidyl side chain and five other, mainly oxygen, donor atoms. Both metal ions are about 23 Å from the carbohydrate binding site and are thought to stabilize the protein conformation. Two histidyl residues have been implicated in the binding of Ni(II) to concanavalin A by titration and chemical modification studies.[254] Since Mn(II) and Ni(II) compete for the transition metal ion binding site of concanavalin A, the two studies appear to be in disagreement.

Carbonic anhydrase, also a zinc metalloenzyme, catalyzes the reversible hydration of carbon dioxide. The two human isoenzymes B and C each contain a single sulfhydryl group that was suggested to be involved in binding Zn(II). However, the single bovine enzyme contains no free sulfhydryl groups, and yet both human and bovine Co(II) enzymes exhibit similar metal ion stability constants and visible absorption spectra.[255] Therefore, the sulfhydryl group in the human enzymes was indicated not to be involved in binding Zn(II) at a relatively early stage in the investigations, and pitfalls that encumbered the carboxypeptidase A studies were, for the most part, avoided. According to an X-ray diffraction study, side chains of histidyl residues at positions 93, 95, and 117 bind the zinc ion in the human C isoenzyme.[256] The fourth ligand about the nearly tetrahedral Zn(II) is water. The single sulfhydryl group is located about 15 Å from the zinc ion. The detailed mechanism of carbon dioxide hydration by carbonic anhydrase remains an unsolved problem, and the specific role of any histidyl residues in the catalysis mechanism is uncertain.[257,258]

Although several metal ions may be substituted for Zn(II) in carbonic anhydrase, only the Co(II) derivative displays enzymatic activity.[259,260] The visible absorption spectrum of the Co(II) enzyme is relatively broad and structured and has been analyzed in terms of a distorted tetrahedral structure.[261] In addition, the absorption spectrum and magnetic circular dichroism of the cyanide complex of Co(II) human carbonic anhydrase B[261] are virtually identical in shape and position on the wavelength scale to that observed for the 1:2 tetrahedral Co(II) complex of *N*-acetylhistidine in 1 *M* base.[143,262] Thus Co(II) in the enzyme takes up the tetrahedral geometry with nitrogen donor atoms indicated for Zn(II) in the X-ray diffraction study. A similar parallel also holds for carboxypeptidase[263] where the tetrahedral Zn(II) when replaced by Co(II) yields an absorption spectrum that may be associated with tetrahedral Co(II).[143] The Co(II) enzymes of both carbonic anhydrase and carboxypeptidase exhibit extremely weak visible circular dichroism relative to the intensity of the absorption spectrum. This weakness signals a tetrahedral geometry about Co(II). Of all the likely geometries about Co(II), only for the tetrahedral one are the d-d transitions that occur in the visible spectrum magnetic dipole forbidden, accounting for the weakness of the circular dichroism. In contrast the magnetic circular dichroism of Co(II) complexes with a variety of geometries exhibit similar ratios of magnitudes of MCD to absorption.

In all the metalloprotein examples known so far where one or more histidyl side chains are involved in binding a metal ion, the other donor atoms are oxygens. This preponderance of mixed complexes with imidazole and oxygen donor atoms is the result anticipated on the basis of the conclusions expressed in section V. Sulfur donors may be similar to oxygen, and there is evidence that some of the copper proteins may contain mixed complexes with sulfhydryl and imidazole side chains. There seems to be no firm evidence of lysyl residue involvement in metal ion binding. The only instances of an amino group serving as donors occur with the terminal α-amino group in the cases such as those described in part B of this section.

It seems certain that, as studies of metalloproteins and biological function of metal ions continue, other instances of metal ion–histidine interactions will be thoroughly documented. Indeed, in view of the unique fea-

tures of this residue among the amino acids with regard to metal ion binding, it will be surprising if histidine is not found to be very widely involved in metal ion binding. At the present time the preceding examples constitute the most completely studied cases. Other systems where histidine–metal ion binding has been implicated are less firmly established. Such preliminary evidence for histidine involvement of metal binding has come from results which are not subject to unambiguous interpretation. For example, epr studies can provide conclusive evidence for nitrogen ligands but often do not further specify the nature of the nitrogen. Studies demonstrating correlation between metal ion binding or enzymatic activity with selective modification of histidine residues may be indicative of histidine involvement in metal ion binding, but alternative interpretations such as loss of conformational integrity on modification can seldom be ruled out without further experimentation. Some examples of systems where only indicative evidence exists are considered in the concluding paragraphs of this section.

Conalbumin and transferrin are closely related Fe(III) binding proteins isolated from egg white and from serum, respectively.[264,265] Both proteins also bind other metal ions, especially Cu(II). Consideration has been given to the possibility that histidine is involved in the metal binding sites in both proteins.[266-270] However, most of the studies on the nature of the metal binding sites have emphasized the role of tyrosine residues so that histidine involvement remains uncertain, as does the detailed nature of the binding site.[271-281] Histidine residues may also be involved in the binding of the iron atoms in the oxygen carrying nonheme protein hemerythrin.[281a]

Alkaline phosphatase, a zinc enzyme, has been studied by epr techniques after replacement of the Zn(II) by Cu(II). The spectra indicate at least three nitrogen ligands, assumed to be histidines, at the metal binding site.[282] Photooxidation studies are also suggestive of histidines at the active site.[283]

There are indications that histidine is a ligand for at least some of the copper atoms in the copper protein ceruloplasmin. Removal of copper to give the apoenzyme results in five to six more titratable groups in the range of pH 7, *i.e.*, in the range of the imidazole ring.[284] Photooxidation, which results in preferential destruction of histidine residues, destroys the oxidase activity of the enzyme and facilitates loss of copper by the enzyme.[285] There is also apparently a correlation between iodination of histidine residues and loss of oxidase activity, when the protein is subjected to iodination.[286] Other studies point strongly to sulfhydryl groups at the copper binding site in ceruloplasmin.[287]

Histidine may act as a ligand in the oxygen carrying Cu(I) protein hemocyanin. The copper binding ability of hemocyanin is diminished in parallel with histidine photooxidation or diazo coupling, from which the inference that histidine is responsible for copper binding can be drawn.[288] Titration data have also formed the basis for the same suggestion.[289] The correspondence noted between the circular dichroism of oxyhemocyanin and the Cu(II) complexes of histidyl containing peptides[290] is probably overdrawn. The visible absorption in hemocyanin requires oxygen and is about 10 times greater than those of the peptide complexes, indicating a quite different kind of interaction. Similar CD spectra are also obtained with Cu(II) complexes of sulfhydryl-containing amino acids.[291] The CD spectrum of mollusc oxyhemocyanin is approximately a mirror image to that of ceruloplasmin. Extensive studies in these and other copper systems have not as yet, however, provided clear evidence for a functional role for histidine ligands in copper proteins.[292]

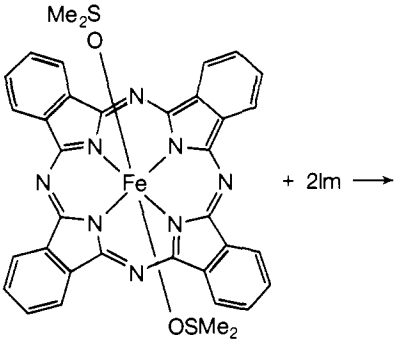
In the Cu(II) and Zn(II) containing enzyme superoxide dismutase (erythrocuprein) electron spin resonance results indicate that three nitrogen donors bind Cu(II), but the technique is unable to distinguish among the kinds of nitrogen donors.[292a] Chemical modification studies suggest that most of these nitrogen donors are from imidazole groups of histidyl residues.[292b]

VII. Imidazole and Benzimidazole as Axial Ligands in Macrocyclic Complex Ions

In this section we discuss the imidazole ring as an axial ligand on metal ions coordinated by relatively rigid macrocyclic rings. The first section deals with a variety of synthetic macrocycles. In the successive sections the corrins and various corrin models are considered, and in the final section the porphyrin or heme family of macrocycles is considered. The macrocyclic ligands are, in general, strong field ligands, and they also impose certain stereochemical restraints on the system. Considerable interest is directed toward the porphyrin and corrins, of course, because of the importance and extensive study of hemoglobin and vitamin B_{12}, the preeminent examples of these structural combinations in biological systems.

A. Synthetic Macrocycles

Although limited by the generally poor solubility in water of phthalocyanine systems, some information on imidazole derivatives of metallophthalocyanines has been obtained. Heating ferrous phthalocyanine with imidazole or a number of its substituted analogs provides bis adducts in which the imidazoles presumably occupy axial positions on iron.[293] Although these compounds have not been studied in aqueous solution, a similar species can be prepared in dimethyl sulfoxide. Ferrous phthalocyanine dissolves in dimethyl sulfoxide to generate a solution presumed to contain two dimethyl sulfoxide molecules in the axial positions (**9**). The kinetics of displacement of these dimethyl sulfoxide molecules with imidazole has been studied.[294] The significant conclusions include the fact that the first substitution is rate determining; *i.e.*, the



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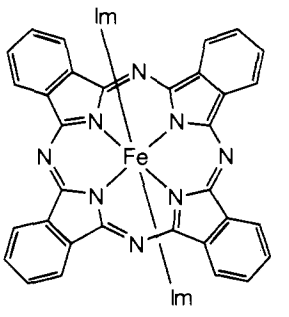

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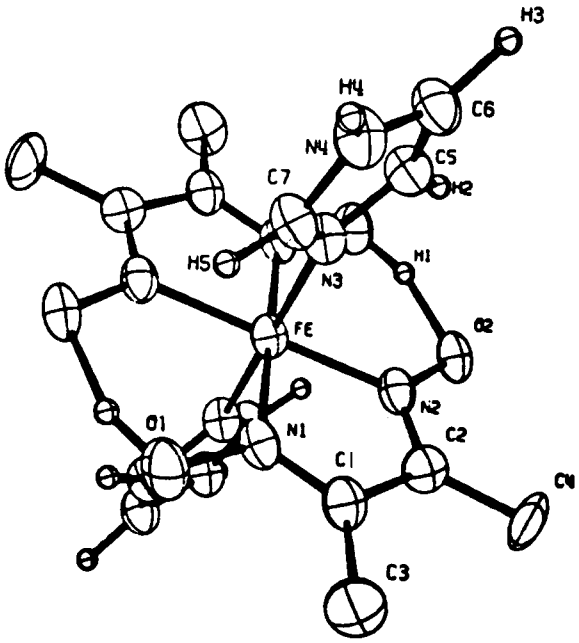


Figure 14. Structure of bis(imidazole)bis(dimethylglyoximato)iron(II). Reproduced from ref 304.

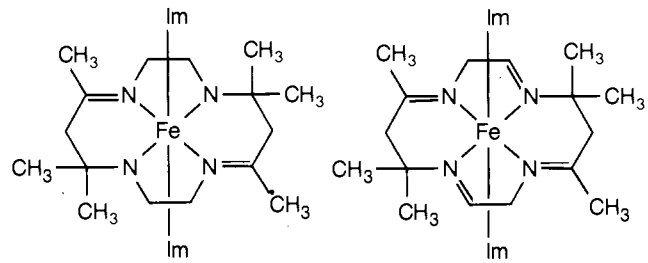
introduction of one imidazole ligand must labilize the remaining dimethyl sulfoxide. The reaction is also characterized by a very small positive entropy of activation, suggesting a dissociative mechanism for the initial substitution. However, kinetic investigations aimed at distinguishing between associative and dissociative substitution mechanisms have not yielded a definitive conclusion.[295] The bis(imidazole) adduct is diamagnetic. As with other cases where comparison with pyridine has been made, imidazole is a weaker π acceptor in the iron-phthalocyanine systems, on the basis of Mössbauer data.[296] An analysis of the electronic spectra of the iron phthalocyanine system with imidazole, pyridine, ammonia, and cyanide as axial ligands has been made. The interpretation given assigns principal importance in determining spectral differences in the series to the σ-donor strength of the ligand,[297] with increasing absorption energy of the major band following the order Py < Im < NH_3 < ^-CN.

The rate of oxidation of low-spin, water-soluble tetrasulfophthalocyanine complexes of Co(II) depends upon the pair of ligands occupying the axial positions.[298] In the presence of imidazole, air effects a relatively rapid transformation to a Co(III) complex while the pyridine complex remains as a Co(II) species. Only qualitative statements concerning rates are presented, and the mechanism of the oxidation is unknown. Among possible reasons for the greater resistance of the pyridine Co(II) complex to oxidation are its greater π-acceptor capabilities and its larger size, which may limit access of oxygen to the metal ion. Investigation of complexes with two different axial ligands should prove informative. The esr spectra of the mono and bis complexes formed by substitution of imidazole and pyridine on tetrasulfophthalocyanine Co(II) in dimethyl sulfoxide have been reported.[299] The spectral parameters are significantly changed by replacing dimethyl sulfoxide by the more basic nitrogen ligands, and imidazole has a more pronounced effect than pyridine.

Complexes of the dimethylglyoxime type with iron in the ferrous state can be prepared if proper precautions against the susceptibility of the compounds toward oxidation are taken.[300,301] The spectra of these substances have been discussed with relation to their applicability as models for members of the iron-porphyrin family.[301] A preliminary report indicating the preparation of the bis(imidazole) adduct of bis(diphenylglyoximato)iron(II) has appeared.[302]

Crystal structure results are available for the axial bis-imidazole Fe(II) complexes with bis(nioxime)[303] and bis(dimethylglyoxime)[304] in the chelate plane. In both structures the in-plane Fe(II)-N distances of 1.94 and 1.91 Å are shorter than the axial ones of 2.05 and 1.99 Å, respectively. Bond distances within the imidazole ring are normal for the latter complex, but the bis(nioxime) complex is reported to contain an unusually long C2-N3 bond of 1.45 Å, indicating a loss of aromaticity in the imidazole ring which seems unlikely. The dimethylglyoxime complex is shown in Figure 14. Mössbauer spectra of the bis(nioxime) and phthalocyanine Fe(II) complexes with a variety of axial ligands suggest that this kind of spectroscopy is apt to be of limited use in establishing the environment of iron atoms in unknown situations.[305]

Recently Busch and coworkers have prepared diamagnetic bis(imidazole) complexes of the macrocyclic iron(II) systems **11** and **12**.[306,307] As yet, however, these systems have been subjected only to preliminary chemical investigations, and not much can be said about any effects the imidazole ligands may have on the chemistry of these systems.



11 **12**

Imidazole complexes of Fe(II) complexed by the 14-membered tetraimine macrocyclic ligand derived from 2,3-butanedione and 1,3-diaminopropane have also been prepared.[307] Two interesting conclusions about the complexes have been drawn from nmr studies. The tendency to form a mixed complex with imidazole as one axial ligand and acetonitrile as the other is about one order of magnitude greater than for formation of the bis complex in acetonitrile solution. In the presence of excess imidazole, the bis complex is formed and separate resonances for free imidazole are seen. Thus it would appear that exchange of complexed imidazole with free ligand is slow on the nmr time scale under these conditions.

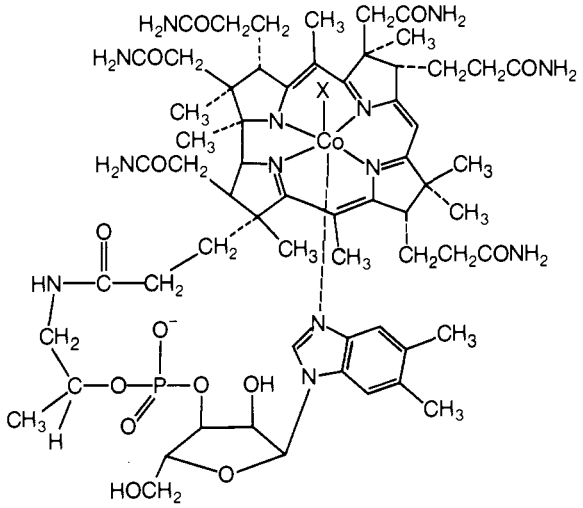
None of the systems described in the preceding section have been subjected to thorough study of their chemical properties. The limited information which has been obtained on these model complexes has not revealed any pronounced special properties associated with imidazole derivatives as axial ligands. By far the most studied of the synthetic macrocyclics is the cobaloxime family. The chemical properties of this class are considered in the following section on cobalamins because of the close relationship between the properties of these two systems.

B. Corrinoids and Corrinoid Models

Major attention has been focused on the benzimidazole ring as a ligand to cobalt because of the presence of 5,6-dimethylbenzimidazole in vitamin B_{12} and related biomolecules. The 5,6-dimethylbenzimidazole molecule, attached by a nucleotide chain to the macrocyclic corrin

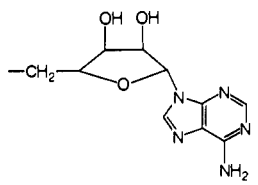
ligand, serves as an axial ligand toward the cobalt ion. The role of B_{12} and related corrinoids in enzymic reactions has been reviewed by Hogenkamp and by Barker.[308] Though vitamin B_{12} refers to the CN^- compound, for historical and preparative reasons, in the biologically active coenzyme CN^- is replaced by the grouping shown in the structural glossary. Extensive discussions of the cobalt coordination chemistry of corrinoids have been published.[309] It is quite possible that the benzimidazole ligand may have a function in modification of the coenzymes reactivity, especially at the other axial position, by virtue of the ability of the Co-N link to be reversibly broken.[310] It has been amply demonstrated that changes in the interaction of cobalt with one axial ligand can significantly alter the geometry of the complex and reactivity of the remaining axial ligand.[309] We will attempt to focus on those studies which define the properties of the benzimidazole ring as a ligand. For purposes of comparison this necessitates some discussion of other axial ligands, but the discussion of other ligands is not intended to be complete.

Structural Glossary for B_{12} Derivatives and Models

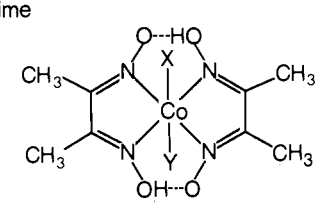


cobalamin, benzimidazole nucleotide is present
cobinamide. benzimidazole nucleotide is absent
B_{12}, X = CN, Co(III)
B_{12r} Co(II)
B_{12s} Co(I)
aquocobalamin, X = H_2O

B_{12} coenzyme, X =



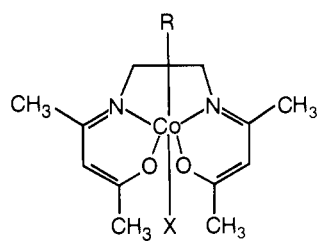
cobaloxime



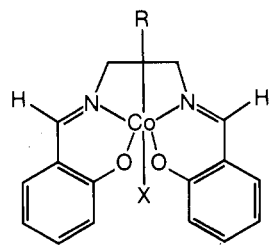
The principle reactivity patterns exhibited by the corrinoids have been elucidated by studies on the natural materials and on model substances. The chemistry of the natural materials have been intensively studied since the early 1960's. The X-ray structure determination[311] of B_{12} and B_{12} coenzyme were landmarks in the application of crystallography to structure determination of biological molecules. Of most direct interest to the present discussion are the structure reactivity relationships elucidated by studies carried out largely by the group at Oxford. In particular, the studies relating to the mutual interaction of the two axial ligands in corrinoids have provided data pertinent to the subject of this review. More recently, a variety of synthetic systems containing cobalt coordinated by macrocyclic ligands have become available. In many respects, these synthetic molecules mimic the reactivity at cobalt found in the natural materials. We will discuss the synthetic macrocycles first and develop some of the information relating to imidazole ligands with examples chosen from model systems. Discussion of studies of the natural corrinoids is deferred to facilitate consideration of these data with respect to mechanisms of the biological reactions. It should be emphasized, however, that many of the general facets of the chemistry of macrocyclic complexes of cobalt were initially observed with the natural materials.

The most studied of the models have been the cobaloximes.[312] The cob(II)aloxime systems are readily obtained from dimethylglyoxime and cobalt(II) salts, and synthetic methods for introduction of a variety of axial ligands are well established. Water molecules which occupy the fifth and sixth (axial) coordination sites can be replaced by other ligands, such as nitrogenous bases, by dehydration followed by addition of the base or, alternatively, by having the potential ligand present in the original preparative solution.[313] Models of B_{12} containing the Co-C bond are prepared from cobalt(II) dimethylglyoxime complexes, a reducing agent, and an alkylating agent, usually an alkyl halide. If no strongly coordinating potential axial ligand is present during the synthesis, a water molecule occupies the sixth coordination site. In the presence of an amine such as pyridine or imidazole, the nitrogenous base occupies the sixth coordination site. The water ligand in the aquo alkylcobaloximes may be replaced by bases such as pyridine.[314,315] Removal of water by drying in the absence of other potential ligands gives dimers derived from pentacoordinate intermediates.[316] Preparation of cobaloximes from cobaltous acetate and dimethylglyoxime in the presence of cyanide ion and such potential ligands as imidazole, benzimidazole, and pyridines produces cobaloximes having one axial cyanide ligand while the other ligand is the heterocyclic base. Similar complexes having halide in place of cyanide are prepared from cobaltous halides, dimethylglyoxime, and the ligand.[317]

Synthetic routes have been developed to other chelated cobalt analogs which have properties generally analogous to the cobaloximes such as **13** and **14**. The most



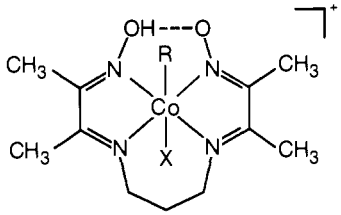
13, Co(acacen)



14, Co(salen)

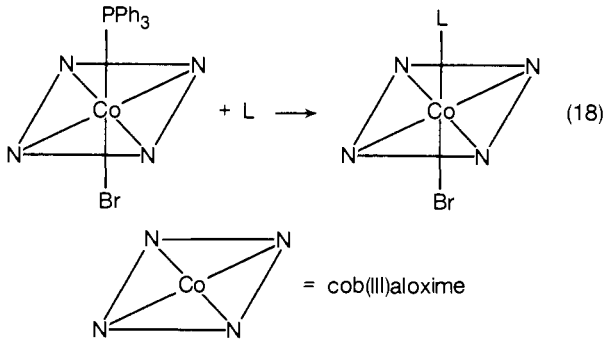
relevant feature of these compounds for the present discussion is the ease with which they can be interconverted between the hexacoordinated and pentacoordinated species by removal or addition of the ligand X.[318] Thus, in aqueous systems hexacoordinated species, X = H_2O, are present but substitution by a heterocyclic nitrogen ligand occurs when the aquo species is allowed to react

with an appropriate amine. The compounds crystallize from noncoordinating solvents as pentacoordinate species. The crystalline hydrated compounds are easily dehydrated *in vacuo*. They are believed to be pentacoordinate in noncoordinating solvents,[318] although some, at least, are dimeric in the solid state.[319] The pentacoordinate derivatives add heterocyclic ligands such as pyridine or benzimidazole readily. A system, **15,** in which the chelating ligand is a monoanion has also been developed and found to show similar properties with regard to exchange reactions of axial ligands.[320]



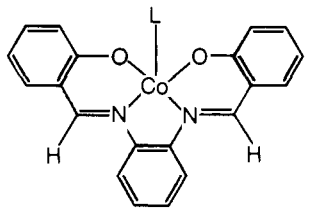
15

The preparative data available would suggest that the imidazole ring is not unlike pyridine in its affinity for alkyl cobaloximes. There is little quantitative information on this point, however. A study of the Co(II) catalyzed ligand equilibration shown in eq 18 established the order of



(18)

binding to be Ph_3P < pyridine < 1-methylimidazole < Bu_3P,[321] but the precise equilibrium constant was too large to be measured in the case of 1-methylimidazole. Equilibrium formation constants have been measured for the pentacoordinate Co(II) system **16.** Here, quantitative



16

measurements were possible, and the K_f for 1-methylimidazole (1.2×10^3) is significantly greater than for pyridine (2.0×10^2). It is also greater than for the more strongly basic benzylamine (1.3×10^2).[322]

The interaction of the axial ligand with cobalt is reflected in the spectral properties of the alkylcobaloximes. A band appears in the region 400–500 mμ and has been assigned as a charge transfer transition.[315] The band shifts to higher energy in roughly the order of increasing σ-donor strength for a variety of ligand types. The order of increasing strength of interaction observed for the nitrogen bases studied is pyridine < benzimidazole < NH_3 < imidazole. This order indicates neither σ-donor or π-acceptor strength alone dominates the nature of the interaction with cobalt but suggests a combination of factors. It is the same order found from stability constant data for the first-row divalent cations (see section III, Table II).

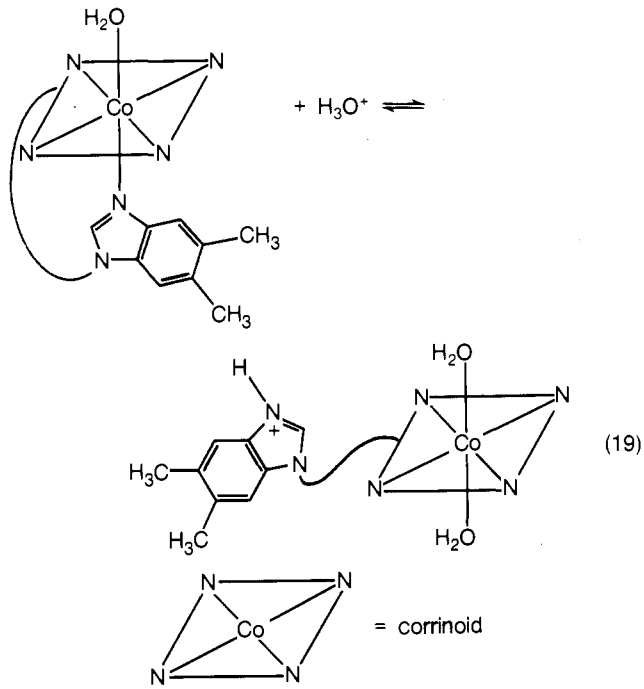
A related matter is the effect of axial bases on the photostability of cobaloximes and cobalamins. In the case of the alkyl cobaloximes, photolysis (aerobic) rates have been reported for the methyl and ethyl systems, and the order of increasing stability as a function of axial ligand H_2O < pyridine ~ benzimidazole < imidazole ~ ammonia is observed, with the overall variation in reactivity being only a factor of 10. In the cobaloximes the effect of the axial base is not primarily structural in nature, but reflects the shifting of the maximum absorption progressively away from the wavelength of maximum intensity of the particular illuminating source which was utilized.[315]

The relative magnitude of the chemical shift ($\Delta\delta$) between the CH_2 and CH_3 of the Co-ethyl groups in ethyl cobaloximes has also been used to assess the donor strengths of a series of bases including imidazole.[323] Ligands, such as pyridine derivatives and imidazole, potentially capable of π-bonding with the cobalt atom give a correlation line distinct from that of saturated bases. Within the group of four aromatic ligands studied, there is a correlation between pK_a and the observed $\Delta\delta$. Pyridine causes a larger $\Delta\delta$, suggesting that the electron density at Co is less in the pyridine complex than in the imidazole complex.

A discussion of the variation of ir bands characteristic of the dimethylglyoximato group as a function of changing the axial ligands has been published.[324] The band shifts have been interpreted as reflecting the extent of donation of electron density to Co by the axial ligand. Although all the band shifts agree with H_2O being a poorer donor than typical nitrogen bases, there are not large differences detectable among such nitrogen bases as pyridine, toluidine, imidazole, and α-picoline. Furthermore, the ordering of these bases varies from band to band. The infrared data do not appear to be sensitive to any differences between the properties of these bases as axial ligands.

The hydrido cobaloximes appear to lie near a stability borderline where the nature of the axial base is quite important. Thus, axial ligands which are believed to form strong π bonds with the metal, trialkylphosphines in particular, permit isolation of hydridocobaloximes. Pyridine complexes have marginal stability near room temperature, but the adducts of the weaker π-acceptor imidazole decompose even at low temperatures.[325]

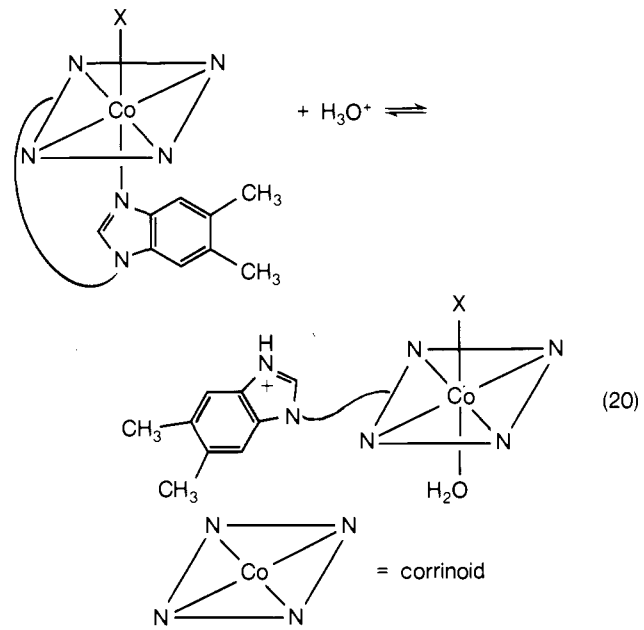
The effect of the nature of axial ligands on a number of molecular properties have been studied in the cobalamin -cobinamide series. The strength of interaction of axial ligands with cobalt coordinated by corrins or corrin models, of course, depends strongly on the electron density at cobalt. Therefore, changes in oxidation state have a marked effect on the strength of binding. In the III oxidation state as in aquocobalamin, a strong ligand-metal bond is present, and the dimethylbenzimidazole ligand is not subject to protonation and displacement until the medium becomes very acidic, the pK_a for the reaction in eq 19 being −2.4.[326] Although the Co(II) form retains the benzimidazole-cobalt linkage, the coordination at the axial position is substantially weakened. Cob(II)alamin is considered to be a five-coordinated species with solvent interacting only weakly at the sixth position.[327-329] The epr signal shows superhyperfine structure ascribed to the coordinating dimethylbenzimidazole nitrogen atom. The pK for protonolysis of the benzimidazole-metal link is



(19)

much higher in the (II) species, being in the range of ~2.5.[330] Coordination with suitable donor bases such as imidazole, histidine, and pyridine occurs with displacement of solvent from the fifth coordination position when the potential ligands are added to methanolic cob(II)inamide solutions. These interactions, too, are detectable by observance of nitrogen superhyperfine coupling in the epr spectra.

The strength of the bonding of the dimethylbenzimidazole unit to cobalt in cobalamins is a function of the trans ligand as judged from equilibrium data for reaction in eq 20. Data on the protonolysis reaction are available for a



(20)

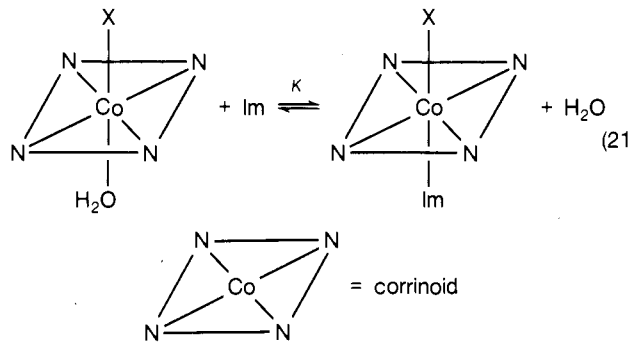
rather wide variety of ligands X.[309,326,331] The position of this equilibrium, of course, reflects the strength of the Co-benzimidazole bond in competition with benzimidazole protonation as a function of the axial ligand X. The protonolysis is increasingly favorable in the order X = H_2O, NH_3, $CH_3N{\equiv}C$, $Cl^- < CN^-$, Br^-, $I^- <$ alkyl. Typical pK values are −2.4 for H_2O, 0.1 for CN^-, and 2.5 for methyl. The series reflects increasing electron density at cobalt weakening the Co-benzimidazole bond. Another

TABLE X. Formation Constants for Imidazole Complexes of Cobinamides (Eq 21)

X	Log K	Ref
H_2O	7.5	347
BzIm[a]	4.6	350
CN^-	4.7, 4.1	347, 505
CH_3^-	1.04	339
$CH_3CH_2CH_2^-$	−1	339

[a] Refers to coordinated benzimidazole nucleotide in aquocobalamin.

measure of this trend is the formation constants for the imidazole complexes of substituted cobinamides (eq 21).



(21)

The reported values are shown in Table X, and this order again suggests that σ-bonding effects are the most important factor in the transmission of the trans effect in the cobalt corrinoids. The larger the charge donated to the central cobalt, the less the tendency to displace water by the stronger donor imidazole. Effects attributable to π bonding between the cobalt and ligand are considered to be minimal.

The strength of benzimidazole coordination is less in branched alkyl than in methylcobalamins. Although incompletely purified, isopropylcobalamin appears to exist with the benzimidazole group uncoordinated, in contrast to primary alkyl cobalamins.[332] A loss of the coordinating benzimidazole is also indicated by spectral data when 5′-deoxyadenosylcobalamin is dissolved in alcohols at elevated temperatures. The loss of ligand in these cases is considered to result in the formation of five-coordinate cobalt derivatives. Spectral evidence suggests that in cyclohexylcobalamin the Co-benzimidazole link is broken.[315] Conversely, hindered alkyl groups are labilized in the presence of excess imidazole, presumably because conformational changes accompanying formation of the axial Co-N bond enhance steric compressions between the branched alkyl group and the corrin ring.[310] The consequences of the facile displacement of the sixth cobalt ligand in alkyl cobalamins, such as 5′-deoxyadenosylcobalamin, with regard to binding of other donors available in biological systems has been discussed by Williams and coworkers.[333] The possible role that the reversible cleavage and formation of the Co-benzimidazole linkage may play in the mechanism of corrinoid-based enzymic reactions has been a subject of considerable discussion, and we will summarize some of these proposals shortly

The pK_a for displacement of the benzimidazole ligand has been measured for a series of halomethylcobalamin derivatives. They fall in the range of 2.5 to 2.1, diminishing with replacement of hydrogen by chlorine or fluorine. As expected the effect of a fluorine is greater than that of a chlorine.[334] Methylcobalamin is reported to have a pK_a of 2.7, but the ethyl and propyl compounds are more subject to protonolysis having pK_a's near 3.8.[335] Although there is some correlation between pK_a for pro-

tonolysis with the electronic nature of alkyl substituents, different correlation lines are found for the $-CH_2X$ and $-CH_2CH_2X$ series. This fact, along with the substantial difference between the methyl and ethyl values, suggests a steric influence on the pK_a for protonolysis.

The C≡N stretching frequencies in cyanocobalamin and cyanocobinamides permit comparison of benzimidazole with water, cyanide, and carbon ligands as to their electronic effect on the cobalt atom.[336] By this criterion the general conclusion is reached that benzimidazole is rather similar to water in its effect. Unlike the compounds with highly polarizable trans carbon ligands, *i.e.*, vinyl, ethyl, and methyl, the C≡N stretching frequency is that of a strongly coordinated (2132 cm^{-1}) cyanide. Frequencies suggestive of a more loosely complexed cyanide (2090 cm^{-1}) are found in the alkyl systems. Assuming again that π-bonding effects are minimal, the correlation between frequency and identity of trans ligand can be considered to reflect primarily electron density changes at cobalt. The ^{13}C chemical shifts have been measured in a series of methyl and cyano cobalamins and cobinamides.[337] The resonance moves *downfield* in the order H_2O > pyridine > dimethylbenzimidazole > cyanide with the total range being about 15 ppm. The trend is opposite to what would be predicted on the basis of a simple argument based on electron density at cobalt, so evidently other factors are dominant.

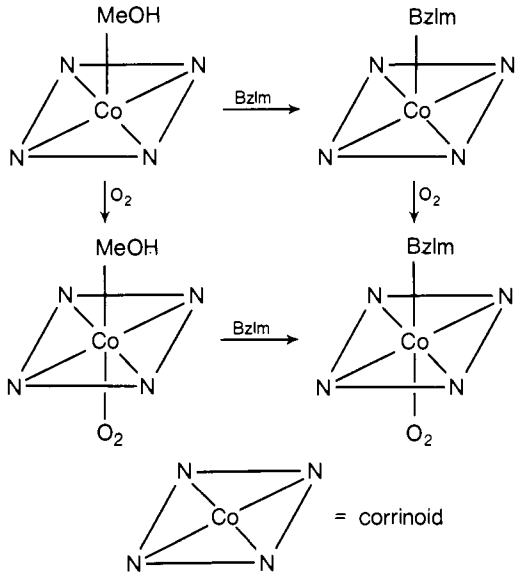
There is some evidence of a relationship between the photolability of the alkylcobinamides and cobalamins and the nature of the coordinating axial ligand. Methylcobinamide is stabilized to photolysis (by a factor of 2 in rate) when imidazole enters the sixth coordination position in place of water. 5,6-Dimethylbenzimidazole is assumed to exert a similar but weaker effect.[338] It has been suggested that the observed photostability of certain cobalamin based holoenzymes can be accounted for by displacement of 5,6-dimethylbenzimidazole at an axial position by a stabilizing histidine residue, but it seems unlikely that this substitution alone could have a very profound effect on the cobalt–carbon bond.

There has also been a polarographic investigation aimed at understanding the interplay of the axial ligands.[339] In basic media, replacement of water by imidazole as the axial ligands in the cobinamide series has little effect on the potential at which reduction occurs, *i.e.*, −0.81 *vs.* −0.75 V for the bis(imidazole) compound *vs.* diaquocobinamide. At pH 12.4 the reduction of aquocobalamin occurs at −1.07 V *vs.* −0.75 for aquocobinamide. However, in neutral and acidic solution the presence of the internal benzimidazole ligand has a much stronger effect on the polarographic behavior. At pH 7.1 aquocobalamin shows its first reduction wave at −0.03 V in contrast to −0.74 for diaquocobinamide. A structural rationalization of this large effect is lacking.

The strong nucleophilicity at cobalt required for certain of the reactions of the cobalamins resides in the Co(I) species, B_{12s}. The effect on reactivity of axial ligands has been probed using cob(I)aloxime models.[340] The recorded[341] nucleophilicity of cob(I)aloxime with benzimidazole as the axial ligand is slightly greater (14.1) than when pyridine is the base (13.8). The reactivity of B_{12s} as a nucleophile is about the same as the reduced cobinamide lacking the Co–benzimidazole link.[340] However, the linkage is probably not intact in B_{12s}. The pmr spectrum of B_{12s} does not show certain shifts of the corrinoid methyl groups which arise as a result of their proximity to the ring current of the benzimidazole ring when it is bound to cobalt.[342] This result does not, of course, exclude the possibility that the Co–benzimidazole link might be reestablished in the reacting complex with a gain in nucleophilicity.

Qualitative effects of the identity the axial ligand on the rate of methylation of Hg(II) by methylcobaloximes have been noted. The imidazole complex is more reactive than the aquo compound.[343,344] Similarly, the rate at which methylcobalamin reacts with mercuric ion is 3000 times greater than the corresponding reaction of methylcobinamide in which the benzimidazole ligand is detached.[345] This is the most striking example of the effect of the identity of the axial ligand on reactivity reported in the corrin system.

Reduced aquocob(II)alamin reversibly binds oxygen, it is assumed with retention of the benzimidazole cobalt bond although the hyperfine coupling is no longer observed.[346] Reversible oxygenation, however, is also observed in analogs in which the benzimidazole ring is not present as an axial ligand. Cobinamides containing ligands such as dimethylbenzimidazole, pyridine, or triphenylphosphine in the fifth coordination position, prepared by displacement of a molecule such as water or methanol, are also susceptible to reversible oxygenation. Oxygenation of methanol cob(II)inamide gives a six-coordinated oxygenated species with a superoxide ion, O_2^-, as one axial ligand and methanol as the other. Addition of benzimidazole results in replacement of the methanol. The introduction of the oxygen ligand shifts the equilibrium in favor of a six-coordinate species. This would be consistent with regarding the cobalt as being in the (III) oxidation state in the oxygenated species (see eq 22).



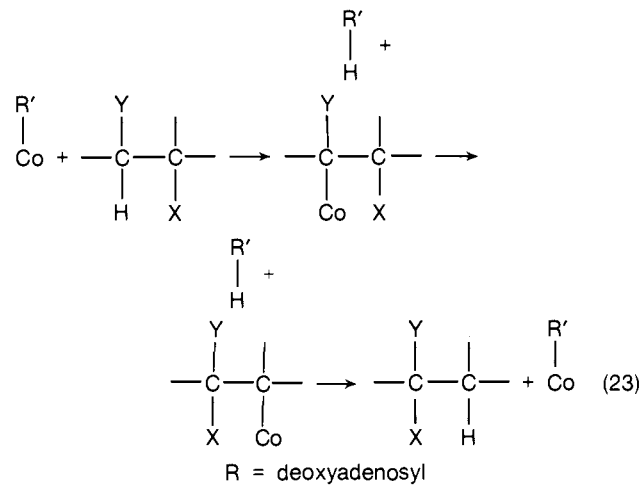
(22)

There are limited kinetic data on substitution reactions on cobalamins and cobaloximes. In general, substitution reactions on Co(III) in cobalamins and cobamides are much faster than corresponding substitutions on simpler Co(III) species. Particularly strong labilizing effects are caused by cyano, sulfito, and alkyl substituents. For example, replacement of ammonia by water in corrinoids having water or benzimidazole as the fifth ligand is characterized by half-lives on the order of hours with the benzimidazole effecting only a sixfold increase in rate.[347] Substitution in the cyano, sulfito, and methylcobinamides, in contrast, is very rapid ($t_{1/2}$ < 2 sec). Model systems studied have included the alkyl 1,3-bis(biacetylmonoximeimino)propanatocobalt system.[348] The rate of introduction of imidazole, pyridine, ammonia, as well as other amines and small anions have been measured. The data suggest involvement of an intermediate complex in the case of imidazole and the other aromatic bases. Kinetics

of introduction of imidazole as a ligand on aquocob(III)-alamin have been reported.[349] The complexation rate is pH dependent, showing a maximum value of 11.1 M^{-1} sec^{-1} at pH 7.4. Dissociation rates calculated from these data and the known formation constant[350] gave values on the order of 6×10^{-4} sec^{-1}. The kinetic data are considered to be inconsistent with a rate-limiting dissociation of aquocobalamin followed by fast binding of the ligand. The conjugate base of aquocobalamin (formed by ionization of the water bound to cobalt) is unreactive toward substitution by imidazole. Kinetic data for substitution of aquocobalamins by a number of small anions are also available.[351,352] The rates are roughly two orders of magnitude greater than for the corresponding substitution by imidazole. The results are consistent with a transition state in which there is loose bonding of both the entering and leaving group to the cobalt. A transition state which is primarily SN1 in character has been proposed for substitution on methylcobaloxime.[353] The structural basis for the remarkable lability of cobalamin complexes relative to other cobalt(III) complexes in general has not been explained.[309,351]

Out of these studies of corrins and corrin models there are clear examples of the following general relationships. (1) A change in axial ligand identity can affect properties which reflect ground-state structural features of the system. These effects seem most often to be related to the σ-donor ability of the ligand. Such changes are usually small on substituting a nitrogen for oxygen but are large on introduction of a carbon ligand. (2) Chemical reactivity at cobalt can be modified by the axial ligand. Again most of the effects seem to be dominated by the electron donor ability of the ligand. There are examples of very large reactivity effects as the result of ligand substitutions. (3) There are appreciable steric or conformational effects observed, especially in the alkyl corrinoids. Alkyl groups larger than methyl have the effect of weakening the bond to the other trans ligand.

It is important to attempt to assess whether these structure and reactivity features can be related to the function of the corrinoids in biological reactions, and we will now summarize the efforts made toward this goal. There is a general understanding of the function of cobalt corrinoids in several enzymic reactions. We will not make an attempt here to consider the individual systems but simply to note the kind of reactivity attributed to cobalt as a basis for considering the role the axial base might play. One important series of enzymes catalyzes a structural rearrangement of the substrate which can be depicted in the general eq 23.[341,354-356] Several of the reactions which fall into this general category have been demonstrated to undergo exchange of H between the substrate and the deoxyadenosyl group of the B_{12} coenzyme. Understanding the nature of the cobalt-alkyl cleavage steps is crucial to description of the reaction mechanism, and this matter has been the subject of intense interest. There are three ways in which the bond could cleave and each has been proposed in one or more systems. It has been proposed[341,355] that a nucleophilic Co(I) species formed by heterolytic cleavage of the Co-C bond in the B_{12} coenzyme might be involved, but objections to this view have been raised.[354,356] The most widely supported mechanism involves homolytic cleavage of the carbon-cobalt bond to form a Co(II) intermediate. Several coenzyme B_{12} dependent reactions have been formulated as involving homolytic cleavage of the Co-C bond generating a radical and Co(II).[357-361] The third cleavage mode, heterolytic Co-C cleavage with formation of Co(III) and the carbanion, has been suggested in the isomerization of methylmalonate to succinate.[362] Still another possibility, based on model systems, is that the rearrangement occurs *via* reversible $\sigma \leftrightarrow \pi$ rearrangement of the alkyl group.[363] The general question of the involvement of the axial base in reactions at the cobalt center has been considered by Brodie.[310,342,364] He has emphasized that a change in axial base coordination can cause significant conformational changes in the entire corrin system. There is ample experimental evidence that in alkylcobalamins the Co-benzimidazole bond is weakened and can easily be cleaved. It has also been proposed[310,339] that formation of a Co(I) intermediate could be accompanied or preceded by cleavage of the Co-benzimidazole linkage. The subsequent attack of the nucleophilic Co(I) on substrate to form a Co-C bond could be accompanied by reclosure of the Co-benzimidazole bond. Proponents of the homolytic mechanism have also considered the possible involvement of the axial base in the Co-C bond cleavages. Evidence[365] that photolytic cleavage of alkylcobalt bonds is retarded when the cobalt-imidazole bond is intact has led to the suggestion that Co-benzimidazole linkage would be broken prior to the homolytic rupture of the 5-deoxyadenosylcobalt bond,[359] but this does not seem to be supported by spectral studies on reacting systems.[358,366]



(23)

R = deoxyadenosyl

In two enzyme systems spectral evidence for formation of a cobalt(II) species on interaction with substrate has been obtained. Addition of an appropriate substrate to 5′-deoxyadenosylcobalamin and ribonucleoside triphosphate reductase results in the rapid generation of a spectrum characteristic of a cob(II)alamin with the cobalt-benzimidazole linkage closed.[366] Experiments with an enzyme of the dioldehydrase family have shown that reaction of substrate is accompanied by generation of cob(II)alamin also with the benzimidazole linkage closed, as identified by its characteristic electronic spectrum.[358] The cobalt(II) compound partially reverts to 5′-deoxyadenosylcobalamin when the substrate is completely consumed. The esr spectrum of the intermediate Co(II) species was also observed.[358] Esr spectra were also recorded in the case of the ribonucleoside triphosphate reductase, but in this case the development of the signals lags the reaction and, therefore, was interpreted as being due to a decomposition product of the active cobalt intermediate.[367]

It is difficult at this time to draw general conclusions about the nature of the participation of the axial base in B_{12}-dependent reactions. No large effects on the reactivity of cobalt are expected on replacement of benzimidazole by the most available potential ligands in biological systems such as water, RNH_2, RCO_2^-, or imidazole. There-

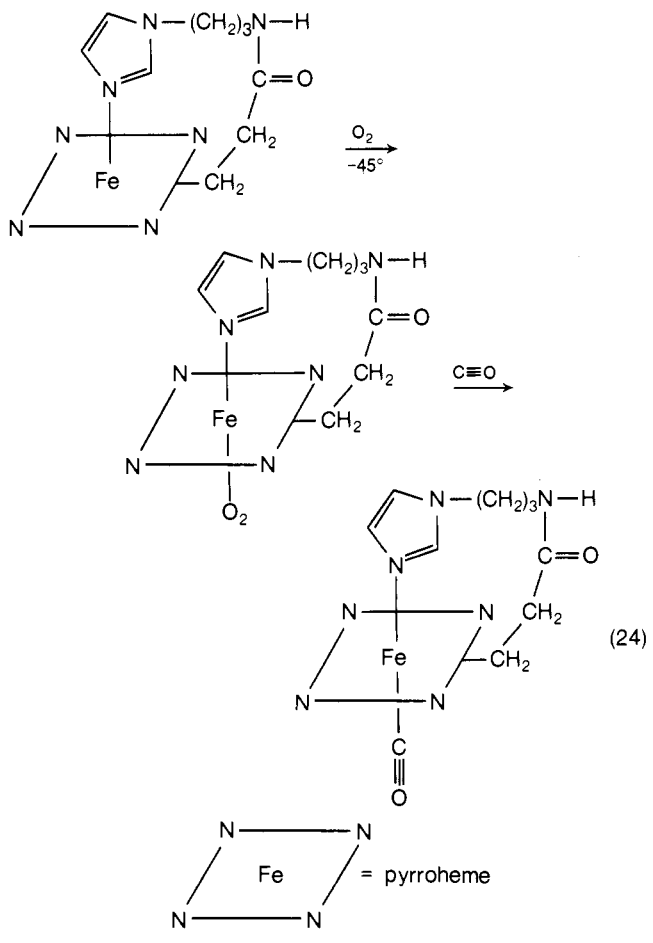
fore, if ligand substitutions are directly involved, it seems likely that reinforcement of reactivity differences by the conformational changes which accompany ligand substitution reactions will be more responsible for reactivity changes than the substitution *per se*. The evidence that the cobalt-base bond remains intact during active function of some cobalt-coenzyme mediated reactions indicates that rupture of the trans axial bond is not obligatory for the coenzyme to function. The strain enforced on the complex as a whole by the protein environment in the enzyme-coenzyme-substrate complex may be a major factor governing reactivity in enzymic reactions.[368]

C. Iron-Porphyrin Systems

The classic example of biological histidine-metal interactions is, of course, the case of hemoglobin[369] and the related myoglobin[370,371] and erythrocruorin.[372] X-Ray structures have in each case revealed bonding of one histidine residue to the porphyrin iron. The existence of authoritative discussions of the nature of the histidine-iron bonding[373] and its role in the heme-protein interaction[374,375] renders extended discussion here unnecessary, although the question of the involvement of the iron-histidine bond in the process of oxygen binding by the iron merits some attention. It is believed that oxygen binding converts the Fe(II) from high spin to low spin with resultant movement of the iron atom from a position as much as 0.8 Å out of the porphyrin plane to a position in which it is in the plane of the nitrogen atoms.[373] The current interpretation of cooperative O_2 binding by hemoglobin envisages this movement as the triggering event which by pulling on the bound histidine results in conformational changes which are transmitted between subunits leading to an increasing avidity to bind oxygen. The details of the conformational changes are considered by Perutz.[374] The cooperative binding so crucial to the physiological function of hemoglobin is then a critical function of the mutual interactions of the iron-bound histidines with the protein chains.

There do not appear to be conclusive direct data available at this time to determine if the particular combination of moderate σ-donor capacity and weak π-acceptor strength found in the imidazole ring represents an optimum for the trans ligand in an oxygen binding system. There are some results which are suggestive, however. Early studies of "hemoglobin models" suggested that the ferroheme-imidazole complex reversibly binds oxygen without oxidation to the ferric complex.[376] It was reported also that both iron(II) protoheme and iron(II) mesoheme in pyridine solution were oxygenated only if imidazole was present. Ordinarily, heme irons detached from their protein component are very susceptible to oxidation to the ferric state. The synthetic hemoglobin model devised by Wang[377] involves 1-(2-phenylethyl)imidazole and reduced heme imbedded in polystyrene. The heme iron is considered to be complexed by the imidazole only in one axial position. This substance has the ability to reversibly bind both carbon monoxide and oxygen. The premises on which the model was conceived included the idea that the low dielectric constant of the matrix would retard oxidation of the iron by mechanisms involving separation of charge and that an imidazole derivative would strengthen oxygen binding.

More recently a heme with an imidazole covalently bound in a position which permits it to act as an axial ligand toward iron has been described. At low temperatures it binds oxygen without oxidation to the ferric state.[378]



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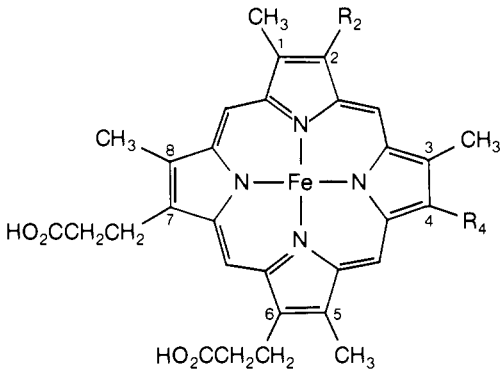
A Structural Glossary for the Hemes



Protoheme	$R_2, R_4 = -CH=CH_2$
Synonyms	Fe(II), heme; Fe(III), hematin
Mesoheme	$R_2, R_4 = CH_2CH_3$
Deuteroheme	$R_2, R_4 = H$
Pyrroheme	$R_2, R_4 = C_2H_5$, H for $CH_2CH_2CO_2H$ at 6
Heme a_2	$R_2 = -CH=CH_2$, $R_4 = -CHOHCH_3$
Heme C	$R_2, R_4 = -CHCH_3$ (S—)
Heme A	$R_2 = CHCH_2CH(CH_2)_3CH(CH_2)_3CH(CH_3)_2$ (OH, CH_3, CH_3); $R_4 = -CH=CH_2$; CH_3 at 8 $= -CH=O$

hemin: hematin with one axial ligand, for example, Cl^- as in hemin chloride

hemochrome: ferrous porphyrin with two axial ligands

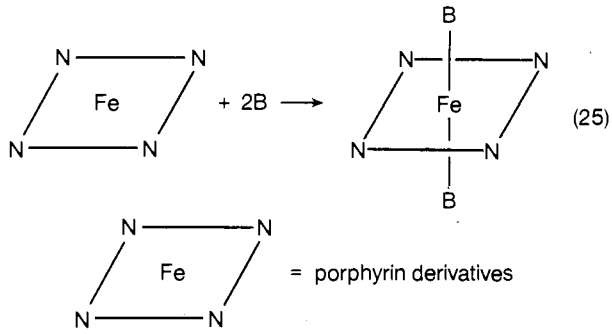
hemichrome: ferric porphyrin with two axial ligands

Oxidation occurs at room temperature. This compound also forms an adduct with carbon monoxide and the spectral properties of both the oxygen and carbon monoxide adducts are similar to those of deuteromyoglobin. See Addendum for additional studies with this system.

A comparison of the effect of axial ligand on the thermodynamics of O_2 binding has been made for cobalt(II) protoporphyrin IX methyl ester in toluene. The strength of O_2 binding was found to be imidazole, 1-methylimidazole > 4-*tert*-butylpyridine, pyridine.[379] Although detailed correlations between ligand properties and oxygen affinity were not found, the imidazoles were more effective in promoting oxygen binding than would be expected if σ-basicity alone were the dominant factor. This suggests that ligand attributes which promote electron density on the reduced state of the metal ion, relatively high basicity and poor π electron acceptor capability, strengthen O_2 binding at the metal ion. Ligand to metal π-donation was also considered to be involved in determining the strength of complexation with oxygen,[379] but the data only indicate a relative ordering of the two heterocycles as donors (or acceptors). Oxygen binding constants for tetra(*p*-methoxyphenyl)porphinatocobalt(II) containing one basic axial ligand have also been determined by an epr technique.[380] In toluene the oxygen affinity order is pyridine ~ piperidine < 1-methylimidazole again indicating that σ-basicity is not the dominant factor. An early preliminary investigation of oxygen uptake by ferrous dimethylglyoxime complexes indicated that histidine, presumably acting as an axial ligand, facilitated oxygen complexation in comparison with other bases such as pyridine.[381] A general tendency toward stronger oxygen binding with increased electron density at the metal has been noted for other metal complexes not so closely related to the biological oxygen carriers.[382]

Qualitative information regarding the effect of axial ligands on the reactivity toward oxygen of heme iron has come from esr studies of iron-tetraphenylporphine model systems.[383] In this system it was found that the Fe(II) compound containing two pyridines as axial ligands was inert to oxygen but that the bis(imidazole) analog was oxidized to the Fe(III) state under comparable conditions. The tetraphenylporphinatocobalt(II) molecule was also included in this study. When pyridine acts as the axial base (the d^7 Co(II) apparently is pentacoordinate in these systems), the molecule acts as a reversible O_2 carrier. A complex is formed with molecular oxygen, but the unchanged Co(II) compound is recovered on evacuation. With imidazole as the axial base, oxygenation is irreversible. The reaction product has not been characterized but is suggested to be a binuclear cobalt complex. Both of these systems indicate a higher reactivity toward oxygen of the metal center when complexed by the imidazole derivative. It has also been reported that the oxidation of cobalt(II) deuteroporphyrin dimethyl ester is promoted by imidazole.[384] The kinetics of reaction with oxygen of the imidazole, 1-methylimidazole, and benzimidazole complexes of protoporphyrin IX dimethyl ester cobalt(II) has been studied. The rates for imidazole and benzimidazole are substantially larger than for 1-methylimidazole. On this basis it was suggested that hydrogen bonding by the N–H group may be involved in the oxygen binding.[385]

Many studies have been directed toward complexes of various iron-porphyrin systems with the intent of using such substances as models for the biologically important heme proteins. Nitrogenous bases including pyridine and imidazole form so-called hemochromes in which the porphyrin iron is complexed with a base at the axial positions. Histidine is also capable of forming adducts of the hemochrome type.[386] Adducts containing dissimilar ligands such as histidine and dimethylformamide or histidine and guanidine can also be obtained. Though extensive quantitative information is lacking, evidence indicates that equilibria favor complexes with two dissimilar axial ligands to a greater extent than expected. Certain of these complexes have been subjected to studies involving comparisons of chemical or catalytic properties of the hemochrome with the parent heme. For example, the histidine hemochromes exhibit both peroxidase and oxidase activity much greater than that observed for hematin itself.[387,388] Such reactivity trends can be considered to model in a rough way the ability of the protein matrix to modify the chemical reactivity of the common heme prosthetic groups on the basis of change of identity or conformation of the axial ligands. It has not yet been demonstrated in detail how structural modification on and around the porphyrin ring can accomplish the changes in reactivity with oxygen and its reduction products necessary to provide heme proteins with such diverse functions as those found in reversible oxygen carriers, mixed function oxidases ($O_2 + S + 2H^+ \rightarrow SO + H_2O$), peroxidases ($S_{red} + H_2O_2 \rightarrow H_2O + S_{ox}$), and catalase ($2H_2O_2 \rightarrow 2H_2O + O_2$). The general subject of the role of the protein matrix in modifying reactivity at a metal ion site has been discussed by Williams in terms of the "entatic state," a strained ground state in which the catalytic site is poised to permit the required reaction to proceed with little further activation.[368]



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Imidazole and dimeric hematin in aqueous solution give a hemichrome product which has certain distinctive spectral properties[389,390] as compared to the pyridine analog. In part at least, the unusual features of this hemichrome spectrum appear to be associated with aggregation of the hemes. The complex is decomposed to free ligand and dimeric hematin by dilution with alkali. The positions of the spectral bands show that the iron has been converted to the low-spin state. The data indicate that the stoichiometry of the reaction is

$$4\text{Im} + [\text{Fe-heme}]_2 \longrightarrow [\text{Fe-heme}]_2\cdot\text{Im}_4 \qquad (26)$$

If it is assumed that each iron accommodates two imidazoles, the dimeric hemichrome must be constructed in such a way that neither axial position on iron is blocked to entry of an imidazole; *i.e.*, the irons must not be stacked directly above one another. Complexes of ferriprotoporphyrin and ferrimesoporphyrin having elemental composition corresponding to 2:1 adducts can be precipitated from solution.[376] A study of the interaction of a number of amines with ferriprotoheme in DMSO indicated that both imidazole and pyridine formed a monomeric ferriheme with the base presumably occupying both the fifth and sixth coordination position on iron, while saturated amines (diethylamine, triethylamine) formed 1:1 complexes believed to be dimeric or oligomeric.[391] Oligomerization and dimerizations of hemes are quite commonly ob-

served. The aggregation is often revealed by characteristic spectral shifts. The state of aggregation is also strongly affected by various detergents. The reaction of heme a with imidazole has been studied by several groups,[392-395] and the species observed are apparently quite sensitive to detergent concentration and charge type. It appears, however, that in solutions containing both ionic detergent and imidazole, monomeric heme-bis(imidazole) complexes are formed.[395]

Considerable attention has been directed at characterizing the spectral changes which accompany hemochrome formation as a function of the identity of the axial ligand. The ultimate goal of such studies is to elucidate the composition of the inner coordination sphere of native porphyrins having distinct spectral characteristics. These efforts have most recently been applied to the various cytochromes, the hemoproteins associated with electron transport and oxidative activity of mitochondria. It has been recognized that there are a number of means by which a perturbation of the iron environment can affect spectral properties, and it has often been difficult to separate the various effects. As in any complex, the separation of the electronic energy levels is perturbed by a change of ligand. In the iron porphyrins, a change of ligand can also change the electronic configuration at iron from high to low spin with attendant spectral changes. Some systems are mixtures of low and high spin, and the axial ligand modifies the ratio of the two forms. In proteins the matters of conformation and metal ligands are closely intertwined. A general change in the conformation of the protein can require a change of ligand at the metal, or displacement of an axial ligand which is essential for maintaining native conformation of the protein chain may result in denaturation of the protein. Finally, many of the porphyrin systems have a tendency for aggregation which is in turn reflected in spectral changes. The interest in histidine, because of its known role as an axial ligand in hemoglobin and certain other hemoproteins, has meant that considerable attention has been focused on it and the parent heterocycle imidazole in such spectral studies. These studies have in themselves not usually been definitive in establishing the identity of heme ligands in proteins but have sometimes provided suggestive data which have subsequently been confirmed by chemical modification approaches or X-ray results. Recently the use of model complexes as spectral models has been extended from small molecule complexes of porphyrins to complexes in a macromolecular environment by studies of the spectral features associated with heme-histidine interactions using poly-L-histidine.[396]

Imidazole rings from histidine are known to be responsible in part for the characteristic spectral features of cytochrome c. It was suggested from a study of the spectra of protoporphyrin derivatives, complexed with imidazole and acetylmethionine, that a combination of histidine and methionine as iron ligands could be responsible for the characteristic spectral features of cytochrome c.[397,398] X-Ray structural data have shown this proposal to be correct by identifying His-18 and Met-80 as the axial ligands on the porphyrin iron.[399] It has also been shown that the histidine-heme linkage is involved in maintaining the compact native conformation of ferricytochrome c.[400]

Imidazole binds to ferricytochrome c, and the binding is accompanied by a spectral change characteristic of displacement of the sulfur ligand (methionine-80) from iron.[401] On reduction the spectrum resembles that of native ferrocytochrome c. The spectral changes which accompany general denaturation of ferricytochrome c are similar to those observed on imidazole complexation.[402-404] It is therefore likely that denaturation of ferricytochrome c is accompanied by substitution of a nitrogen ligand for the methionine residue which is necessary for the spectrum characteristic of the native enzyme.[404] The kinetics of substitution of imidazole for methionine-80 in cytochrome c have been studied and compared with similar data for pyridine and for cyanide and azide ion.[405] The data are considered most consistent with a SN1 limiting mechanism in which cleavage of the iron-sulfur bond and formation of a reactive iron species is rate determining.

Cytochrome c can be modified by alkylation with bromoacetate under conditions which result in alkylation of two methionine residues, one of which is the methionine-80 which occupies a coordination site on iron in the native enzyme.[406] The modified cytochrome reversibly forms an oxygen adduct of modest stability and an imidazole complex with a formation constant of about 10^3 M^{-1}. The imidazole complex is rapidly oxidized to the ferric state by O_2, in contrast to the behavior of the modified protein in the absence of imidazole.

In cytochrome b_5, a cytochrome isolated from liver microsomes, the porphyrin moiety is protoheme. It has been suggested that heme binding is through histidines,[407] and this suggestion has been supported by X-ray data on calf liver cytochrome b_5 which shows His-39 and His-63 coordinated to the heme iron.[408] It is hypothesized that histidines are the axial ligands in cytochrome b_{562}, one of the smaller cytochromes of the *b*-type. The peptide chain of this cytochrome contains only two histidines.[409] Evidence that at least one histidine serves as an iron ligand in cytochrome b_2 has been obtained by photooxidation studies which showed that destruction of two histidines in the apoenzyme parallels loss of heme binding ability.[410] A tentative suggestion has been advanced that histidine serves as the fifth ligand in cytochrome P-450 cam, a cytochrome of the mixed function oxidase type.[411] There is considerable evidence that an alkyl sulfide group, presumably cysteine, is the sixth iron ligand, at least in the ferric oxidation state of cytochrome P-450.[412-414] In general, there are strong indications that the porphyrin iron of the cytochromes usually contains at least one histidine bound to each iron. The description of the function of the cytochromes on a molecular basis remains incomplete, and it is not possible to indicate how intimately the imidazole ring might be associated with their electron transfer function.

There has also been spectral[415,416] and chemical modification data[417] indicating the presence of histidine-heme iron coordination in peroxidase and catalase. The presence of a nitrogen superhyperfine splitting in the epr of the NO complex of ferrous peroxidase has also been taken to indicate the possibility of a iron-histidine link.[418] No such splitting is seen in catalase. The identity of the axial ligands in neither peroxidase nor catalase can be considered to be established beyond question, although the evidence that a nitrogen ligand occupies an axial position in peroxidase is good.

Imidazole can complex in some instances with heme iron which is present in association with the native protein. The case of the imidazole complex of cytochrome c was considered earlier. The redox potential of myoglobin is modified by addition of imidazole,[419] presumably by complexation at iron.[420] There is epr evidence that imidazole complexes with cytochrome c_3.[421] The hemoprotein, P-450, which is associated with the hydroxylating and demethylating activity of microsomes also forms a complex with imidazole as well as with other nitrogenous

ligands,[422-424] resulting in characteristic spectral changes.

The nature of the bonding of the imidazole ring to heme iron is presumably similar to the bonding of aromatic bases to transition metal ions in general. There is both a σ-donor and π-acceptor (back bonding) character associated with the imidazole ring. As discussed in section II, the most studied ligands for comparison are pyridine and its derivatives.[425] Imidazole and imidazole derivatives have a comparable affinity for the iron(III) and iron(II) porphyrins.[426,427] In contrast, pyridine and nicotinamide are more strongly bound in the Fe(II) state than the Fe(III).[426-428] The results are consistent with the idea that for imidazole complexes the σ-donor property is more important than π-acceptor capacity in determining the strength of binding while pyridine, because of its greater π-acceptor capability, interacts more strongly with Fe(II), the better π donor. A Mössbauer study, in which ferric and ferrous porphyrin complexes containing axial imidazole or pyridine ligands were compared, supports the conclusion that pyridine binds more strongly with ferrous iron than does imidazole and indicated that imidazole has a stronger affinity for Fe(III) than does pyridine.[429] A π-donation from imidazole to Fe(III) was also invoked as the stabilizing influence in the ferric system, but the greater σ-donor capacity of imidazole could also be responsible.

The bis(imidazole) complex of tetraphenylporphinatoiron(III) has been studied in various organic solvents.[430] No evidence for a mono imidazole complex was found, establishing that the introduction of the first imidazole increases the affinity of the metal ion for the second imidazole molecule. This is believed to be the result of the conversion of the Fe(III) from high spin to low spin on introduction of the first nitrogen ligand. The equilibrium in aromatic solvents has been interpreted in terms of a nondissociating ion pair. The measured formation constants, β_{21}, are, however, a function of total imidazole concentration. Two independent studies in acetone produce divergent values and interpretations for the formation constants. Coyle, Rafson, and Abbott give 6.6×10^5 M^{-2} for formation of the bis(imidazole) complex as a nondissociating ion pair while Duclos gives 5.5×10^4 M^{-1} under similar conditions but with the equilibrium formulated as involving chloride ion dissociation. The infrared spectrum of the crystalline perchlorate salt of bis(imidazole)tetraphenylporphinatoiron(III) has been described.[431]

The bis(imidazole) adduct of tetraphenylporphinatoiron(II) has also been prepared.[432] In contrast to imidazole, 2-methylimidazole forms a complex in which only one imidazole molecule is bound to iron.[432] It is believed that the 2-methyl substituent would interfere sterically with the porphyrin ring in a bis adduct having iron in the plane of the ring. The complex is instead believed to be five-coordinate, but this has not been established with certainty since the isolated crystalline material contains 1 equiv of ethanol which could conceivably be coordinated.

The proton nmr spectra of the bis(imidazole) adducts of several synthetic ferric porphyrins have been studied, and the mechanisms of the shifts caused by the low-spin d^5 Fe(III) have been analyzed.[433] The results indicate imidazole does not have a strong π-donor interaction with Fe(III).

Formation constant data for the introduction of one axial ligand into tetra(*p*-methoxyphenyl)porphinatocobalt(II) is available for a series of pyridines, imidazoles, and saturated bases.[434] In general the strength of binding is imidazoles > pyridines > saturated bases. However, in the cobalt(II) complex of protoporphyrin IX dimethyl ester, the formation constants for imidazole and 1-methylimidazole 1:1 complexes in toluene are quite similar to the formation constant for pyridine.[379]

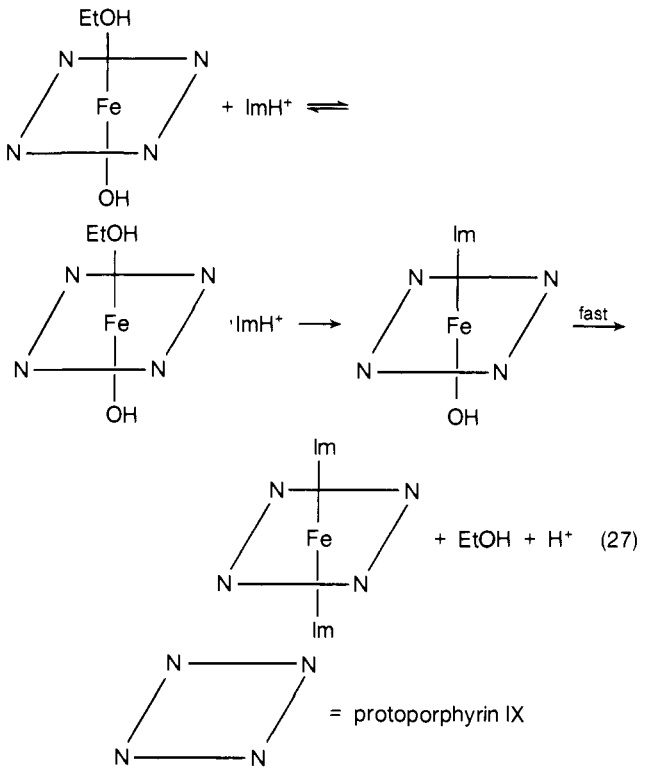
The interaction of the imidazole ring with heme iron has also been studied by epr techniques. Imidazole is apparently capable of two distinct types of interaction with NO-ferroheme.[435] In nonpolar solvents the epr signal arising from the delocalized electron is similar to that of pyridine-type bases. These spectra indicate a strong interaction between the iron and base through π as well as σ orbitals. In polar solvents a modified spectrum with characteristics suggestive of axial symmetry is observed. A detailed explanation has not been offered for this observation, but it has led to the suggestion that changes in the conformation of interaction between imidazole and iron could be capable of modifying electronic structure in the vicinity of iron significantly. The above epr results on the isolated heme system have been used in an attempt to clarify the interpretation of epr data available on nitric oxide complexes of hemoglobin[436] and ferrocytochrome c.[437] A conformational change involving tilt of an axial histidine has been considered as a possible cause of the unusual rhombicity detected in the epr spectrum of high-spin cytochrome P-450.[413] However, it was felt that interaction of a portion of the porphyrin ring with an aromatic group from the protein matrix was probably the cause of the observed effect. Additional characterization of histidine-iron binding in hemoproteins may be obtained by endor techniques. This technique has recently been applied to metmyoglobin.[438]

A carbonylated ruthenium(II) complex of mesoporphyrin has been prepared (a low-spin system). It complexes readily with imidazole in nonpolar solvents such as benzene.[439] The binding is quite weak and is reversed in donor solvents such as tetrahydrofuran or methanol. The proton magnetic resonance signals assigned to the imidazole ring in this complex were thought to show a rather curious temperature dependence. The signals assigned to the 4- and 5-protons merge above 100° but not to the average chemical shift position which would be expected for a simple rapid exchange of two species. An incomplete explanation based on "movement of the imidazole through several different orientations with respect to the porphyrin ring" was advanced.[439] However, the 4-*tert*-butylpyridine complex of a similar ruthenium(II) carbonyl tetraarylporphine has been shown to exchange with uncoordinated 4-*tert*-butylpyridine under similar conditions, and a reexamination of the imidazole system was suggested.[440] Faller and Sibert[441] have revised the signal assignments of Tsutsui, *et al.*, and concluded that the exchange process is in fact intermolecular. The possibility that imidazole derivatives can adopt an unconventional mode of bonding in ruthenium porphyrins, therefore, remains to be demonstrated.

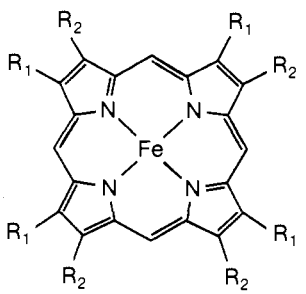
The structural details of imidazole-heme iron bonding have been approached by X-ray studies on crystalline imidazole-porphyrin complexes. A crystal structure of bis(imidazole)tetraphenylporphinatoiron(III) chloride, a low-spin system, is available.[442] The structure is shown in Figure 15. The two axial imidazoles are skewed with respect to one another and, because of differing nonbonded interactions with the porphyrin ring, are at slightly different distances, 1.96 and 1.99 Å, from the iron atom. This study confirms the expectation that the iron would be coplanar with the porphyrin nitrogens when in a hexacoordinate low-spin state. The iron is only slightly displaced (0.009 Å) from the plane of porphyrin nitrogens. The preference for five-coordinate noncoplanar iron in

high-spin ferric systems has been demonstrated by the structures determined for methoxyiron(III) mesoporphyrin IX diethyl ester,[443] chloroiron(III) tetraphenylporphine,[444] and chlorohemin.[445]

The kinetics of binding of imidazole to sperm whale metmyoglobin has been studied by temperature-jump techniques.[446,447] The rate of imidazole binding to the myoglobin is much smaller than for molecules such as oxygen and carbon monoxide. It is believed that a substantial conformational change accompanies accommodation of imidazole into the iron coordination sphere. There have also been kinetic studies involving temperature-jump techniques on the complexation of imidazole with isolated porphyrin systems, specifically ferriprotoporphyrin IX.[448] The rate of displacement of an ethanol molecule from an axial site by imidazolium ion was found to be faster than the corresponding substitution by neutral imidazole. An ion pair mechanism in which the imidazolium ion protonates the departing ethanol was proposed. Excess imidazole was present, and under these conditions the second substitution is sufficiently fast that no intermediate could be detected spectroscopically. Alternatively, the term in imidazolium ion may also be accounted for by free imidazole attack on the aquo hemin.



Contrasting results were obtained with a synthetic water-soluble Fe(III)-porphyrin system (**17**) although the qualitative conclusion was again reached that imidazole substitution on iron in porphyrin environments is very



17, $R_1 = CH_3$
$R_2 = (CH_2)_2NH(CH_2)_2NH_2$

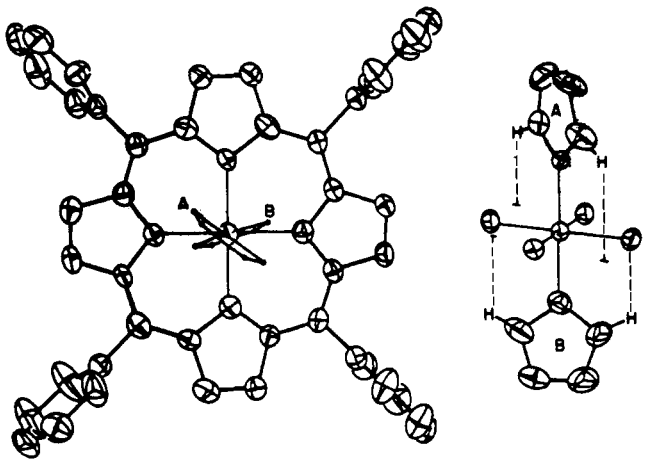


Figure 15. Structure of bis(imidazole)tetraphenylporphinato iron(III) chloride. Reproduced from ref 442.

fast.[449] With this positively charged porphyrin, the neutral imidazole molecule is the only reactive form of the ligand, and the stable complex contains one imidazole and one water as axial ligands. A bis(imidazole) complex could not be observed. Nmr line broadening studies have provided estimates of the kinetic parameters of exchange of 1-methylimidazole at ferritetraphenylporphyrin and ferrioctaethylporphyrin. In chloroform solution at room temperature, a rate of constant of ~60 sec^{-1} was reported with an E_a ~17 kcal/mol. The analogous exchange in ferrioctaethylporphyrin was significantly more rapid.[450]

It is generally assumed that an imidazole labilizes the trans ligand in porphyrin complexes.[451,452] The quantitative measurement of this effect has been difficult, however. The numerous kinetic studies carried out on substitution reactions of hemoproteins usually cannot separate effects due to the specific influence of the imidazole ring from those of the protein structure as a whole, nor do the natural substrates permit comparison with analogs in which the imidazole ring is replaced by a different chemical entity. The few kinetic studies on porphyrin model systems indicate that in formation of bis(imidazole) complexes introduction of the second imidazole is rapid, but these studies do not permit comparison with other axial ligands. It will be recalled that the cobalamin studies have not revealed a large labilizing effect of benzimidazole relative to water at Co(III) centers. The rapid reaction with the second imidazole molecule may be the result of the change in coordination geometry and spin state at the metal center which results from incorporation of nitrogen ligands.[430]

Imidazole catalyzes introduction of Zn(II) into a modified water soluble porphyrin, but the details of the catalytic process are unknown.[453]

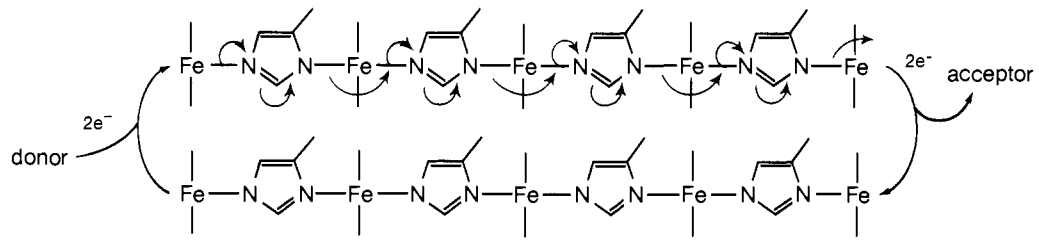
The current state of understanding of the role of axial ligand-iron interactions in the biological function of hemoproteins can be summarized briefly. The role of axial ligands in modifying the spectral and magnetic properties of the iron-heme system is well documented. Also firmly established is the importance of the iron-histidine bond in coupling protein conformation to the ligation and oxidation state of heme iron. The role the imidazole ring of histidine may play in modifying chemical reactivity at the iron atom is much less well defined. Although there are indications that the imidazole ring is a favorable ligand for oxygen binding by iron, and that it can, in certain cases, strongly effect the redox behavior of the metal ion, the data seem insufficient to permit conclusions about the details of any role that histidine ligands may play in determining the course of reactions between iron

and oxygen which occur in hemoproteins. Studies on model systems in which the effect of the ligand can be studied in the absence of the protein promise some insight into this question. The Addendum refers to one recent study which demonstrates that the imidazole ring is markedly superior to the pyridine ring in facilitating O_2 binding by heme iron.

VIII. Imidazole and Histidine as Ligands in Redox Processes

The importance of metal ions coordinated to histidine in biological redox processes, as illustrated in particular by the cytochromes, brings to the fore the following question. Does the imidazole ring possess any unique structural features which make it crucial to metal ion mediated redox process, or is its apparent widespread involvement simply a result of its general suitability as a metal ion ligand? This section focuses on imidazole–metal ion systems in redox processes in the hope of providing information relevant to the above question.

In 1965 Urry and Eyring[454] put forward a model for electron transport in the cytochromes which proposed that the imidazole rings mediated electron transfer by alternately binding to each of two adjacent iron porphyrins, thereby effecting electron transfer. A related model involving quinones was constructed to account for the coupling of phosphorylation with electron transport.[455] These models suggest that the symmetrical nature of the imidazole ring can minimize the activation energy involved in



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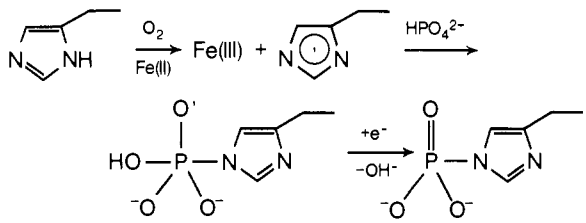
electron transport. Either model would seem to require direct intermolecular interactions between several cytochrome molecules in order to permit the required chemical arrays. Specifically, they would require that the axial histidines be at the surface of the protein to facilitate interaction with the next component of the chain. Indeed, both sides of the porphyrin would need to be accessible to an adjacent protein bound histidine. Although the functioning of the mitochrondial electron transport system is known to depend critically on membrane structure, which presumably maintains a proper array of molecules, the X-ray structural work which has been accomplished since the imidazole pump model was suggested has generally placed the iron and the attached histidine inside the protein structure, although an edge of the porphyrin ring is exposed in both cytochrome b_5[408] and cytochrome c.[399] At least in these two relatively small cytochromes, the buried nature of the axial histidine residues suggests that the "imidazole-pump" mechanism of Urry and Eyring would be unlikely. A second model for oxidation phosphorylation which intimately involves the imidazole ring has been formulated by Wang.[456] The proposal is that an imidazole ring from histidine is activated by oxidation with O_2. A cytochrome Fe and the imidazole ring each act as one-electron donors. The imidazolyl radical thus generated is presumed to be capable of reacting with inorganic phosphate, eventually giving a phosphorylated imidazole derivative which subsequently effects phosphorylation of ADP to ATP. The model has been extended to include photoinduced phosphorylation.[457] The detailed molecular basis of the mechanism of electron transport remains unknown, and it remains to be seen whether either model has any validity (see eq 29).



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There have been a limited number of studies on simpler imidazole–metal ion systems aimed at characterizing their redox behavior. We shall discuss first the effect of imidazole ligands on Cu(I)–Cu(II) redox systems. A variety of enzymes involved in oxidative process are known to involve copper although considerable uncertainty remains with regard to the immediate environment of the metal ion.[292]

The stability constants of Cu(I) are greater than those of Cu(II) for the 1:1 and 2:1 complexes with NH_3, imidazole, and pyridine listed in Table II and for benzimidazole.[101,102] This result is a general one, and for a variety of monodentate nitrogen donor ligands the standard reduction potentials of the Cu(II)/Cu(I) couple are more positive than for water.[101,458] The potential increases about 0.09 V for each nitrogen donor added up to two. With amino acids such as histidine that chelate with Cu(II), Cu(I) prefers linear coordination so that it is relatively destabilized, and the standard reduction potential becomes negative. Variations of the potential with changes in coordinating ligands are important for delineating the copper binding site in proteins and the role of the Cu(II)/Cu(I) couple in the mode of action of copper containing proteins in which the metal ion undergoes a change of valence state in performing its function.[459]

The kinetics of oxidation of $Cu(Im)_2^+$ and of the analogous 1-methylimidazole complex have been studied, and rate laws were found to be first order in $Cu(Im)_2^+$, excess Im, and O_2.[460] Analysis of the kinetics has led to the conclusion that oxidation occurs primarily through a species $[Cu(Im)_3O_2]^+$. The preference for a four-coordinate intermediate can be understood as a requirement for attaining a coordination geometry suitable to the metal ion in its oxidized state, *i.e.*, four-coordinate in the case of Cu(II). The redox equilibrium which occurs in solutions containing Cu, O_2, I^-, and Im has been studied by epr techniques. High Im concentration favors the (II) state by displacing the equilibrium in the direction of the tetrasubstituted $Cu(Im)_4^{2+}$.[461]

The oxidation by molecular O_2 of Cu(I) complexes containing histamine, histidine, carnosine (β-alanyl-L-histidine), and histidylhistidine have been studied.[462] The two dipeptide complexes are substantially more reactive than the nonchelated $Cu(Im)_2^+$ species and much less reactive than the histidine complex. Presumably chelation, which favors the Cu(II) state over Cu(I), is the primary structural feature responsible for the rate enhance-

ment. An earlier, brief description of the histidylhistidine-Cu(I) system led to the conclusion that the histidine ligand was oxidized during the reaction.[463]

Copper(II) complexes catalyze the decomposition of H_2O_2: they function as catalases and peroxidases.[464] The catalatic activity of the Cu(II) complexes of imidazole,[465] histamine,[466] histidine,[467] and other amines[468] has been studied kinetically over a range of conditions by one set of authors, and the results have been compared.[468] Maximal activity is achieved with two cis nitrogen donors. Only the 2:1 imidazole-Cu(II) complex is active; the 1:1, 3:1, and 4:1 complexes are inactive. The reaction rate is proportional to the product of the complex and the concentration of HOO^-. Apparent rate constants in M^{-1} sec^{-1} at 25° for the 2:1 complex of imidazole and 1:1 complexes of other ligands are as follows: histidine, 130; imidazole, 530; diaminoethane, 700; 1,3-diaminopropane, 1000; and histamine, 1300.[468] It is suggested that this order supports the view that reduction of Cu(II) to Cu(I) occurs during the reaction. A possible rate-limiting step is considered to be electron transfer from bidentate HOO^- to Cu(II) in a mixed complex with O-O bond rupture due to the widening of the chelate bond angle in the Cu(I) complex.

Often H_2O_2 decomposition is accompanied by oxidative degradation of the ligands bound to Cu(II); peroxidase activity occurs.[464] This degradation has not been closely investigated in imidazole systems. Histidine and histamine are known to be degraded by H_2O_2 when complexed to Cu(II), but the products have not been characterized.[137]

A variety of copper complexes are capable of catalyzing oxidation of substrates such as diphenols, aromatic amines, and ascorbic acid. Only a qualitative comparison of imidazole with some other pyridine-type ligands appears to have been published.[469] The oxidation by oxygen of ascorbic acid, hydroquinone, tetramethylphenylenediamine, and some related substrates catalyzed by Cu(II) complexed to poly-L-histidine has been studied.[470] Copper bound to the polypeptide is a more active catalyst toward neutral and anionic substrates than copper complexes of imidazole and histidine. This is attributed primarily to the electrostatic charge present on the polypeptide. The oxidant, molecular oxygen, is reduced to hydrogen peroxide. Undoubtedly several copper-containing proteins which catalyze similar reactions will be found to have histidyl side chains bound to the metal ion.

Copper (bis)histidine is a catalyst for oxidation of cytochrome c by hydrogen peroxide[471] and oxygen.[472] The rate law is first order in each reactant, but there has been insufficient study to establish a mechanism. The reaction system is complicated by apparent reduction of the oxidized cytochrome as the concentration of hydrogen peroxide is depleted.

Another area of redox chemistry in which imidazole-metal systems have received some study is the reversibly formed O_2 adducts of Co(II) in which histidine and related molecules have figured prominently, but not by any means exclusively, as ligands.[473,474] The discovery of the reversible oxygenation of high-spin, octahedral bis(histidinato)cobalt(II) in neutral and basic solutions to yield a brown, diamagnetic, binuclear oxygenation complex was made nearly 30 years ago.[475,476] The strong charge-transfer absorption giving rise to the brown color (yellow in dilute solutions) obscures the d-d bands, but they are revealed in circular dichroism which indicates that the complex contains Co(III).[473,477] Most of an electron is transferred from each Co(II) to the bridging oxygen so that the so-called oxygenated complex is more properly

$$2Co^{II}L_2 + O_2 \rightleftharpoons [(L_2Co^{III})^+O_2^{2-}(Co^{III}L_2)^+] \quad (30)$$

described as a peroxo complex of Co(III) (eq 30). This conclusion is supported by an X-ray structure determination of decaammine-μ-peroxo-dicobalt where the O-O bond length corresponds to that of peroxides.[478] The brown color is then assigned to a peroxo to Co(III) electron transfer transition. Only the infrared spectrum and no X-ray structure is available for a crystalline peroxo-bridged complex with histidine.[479] It is generally believed that one histidinato carboxylate on each Co(III) has become detached to permit coordination of the peroxo bridge.

The peroxo formulation of the binuclear complex does not exclude easy accessibility to the Co(II) state by the reverse of the above equilibrium reaction.[473] Thus the binuclear peroxo complexes are more labile than most Co(III) complexes. With histidine the reverse reaction takes place upon bubbling pure nitrogen through the solution. With other ligands that yield protons on the right-hand side of eq 30, it is also necessary to add acid as well as nitrogen to reverse the reaction. In all cases addition of sufficient acid alone reverses the reaction because ligand protonation and formation of aqueous Co(II) occurs.[473] The integrity of the O-O bond is preserved upon cycling oxygen through the reversible reaction.[480]

For histidine the equilibrium constant for formation of the binuclear peroxo complex of Co(III) is substantial and characterized by unusually large negative enthalpy and entropy changes.[481] The reaction proceeds by way of a rapidly formed mononuclear oxygen adduct which for histidine does not build up to a detectable extent.[482,483] For other ligands where the 1:1 adduct may be studied directly, most of an electron is again transferred to the metal ion so that the complex is most simply described as a mononuclear superoxo (O_2^-) complex of Co(III).[346,484-486]

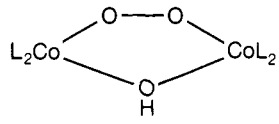
Attempts to apply the superoxo Co(III) description of the reversible cobalt ion oxygen carriers to describe oxyhemoglobin as a superoxo complex of low-spin Fe(III) with two unpaired but antiferromagnetically coupled electrons[487] seem overdrawn. Loss of a t_2 electron from low-spin Fe(II) seems energetically unfavorable. Compared to other axial ligands in heme systems the superoxide ion is expected to yield a predominantly high-spin Fe(III) complex.[488] In addition, carbon monoxide, which is not an electron acceptor, combines with hemoglobin but not with the bis(histidinato)cobalt(II) complex.[476]

The strong one-electron oxidant Ce(IV) converts the binuclear, peroxo, bis(histidinato)cobalt(III) complex to a green, binuclear, superoxo complex.[477,489,490] The absorption band at 680 nm and the electron spin resonance spectrum indicate that the bridging ligand is aptly described as the superoxide ion, O_2^-. In contrast to the peroxo complex, the superoxo complex is stable in strong acid.

Molecular oxygen also reacts with mixed ligand systems containing imidazole and alanine with Co(II) to give brown solutions typical of peroxo complexes.[491] Without alanine imidazole alone with Co(II) is unreactive. Substituted pyridines are less effective than imidazole in increasing the absorption intensity near 370 nm. Oxidation by Ce(IV) yields superoxo complexes as indicated by solutions with absorption maxima near 700 nm. Both peroxo and superoxo complexes are formulated as binuclear.[491]

Upon admission of oxygen to a solution containing the 2:1 histamine complex of Co(II), one mole of acid is released for each mole of binuclear complex that is formed. This odd number of equivalents of acid for each

two moles of metal ion is due to formation of a hydroxo bridge in addition to the peroxo bridge linking the two Co(III) so that the binuclear complex is dibridged.[473] Hydroxo bridges form with other ligand systems unless in-



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hibited by steric effects or lack of suitable coordination sites about the cobalt ion.

Oxygenation of solutions containing 2:1 molar ratios of histidine to Co(II) yields different products at pH 9 and in 1 *M* base, as indicated by ultraviolet absorption spectra.[492] The products are interpreted as monobridged and dibridged binuclear peroxo complexes, respectively, formed from octahedral and tetrahedral (four nitrogen donor) Co(II) complexes.[143] The dibridged complex, containing both peroxo and hydroxo bridges, may also be formed by adding sufficient base to a solution containing the monobridged species so that the solution becomes about 1 *M* in base. Addition of acid does not regenerate the monobridged species, but addition of excess acid does destroy the complex and give protonated ligand and Co(II) with release of oxygen.[477,492,493] The dibridged complex also forms slowly in solutions less basic than 1 *M* base.[493] Excess EDTA decolorizes solutions containing the monobridged complex more rapidly than the dibridged species, indicating that the addition of a hydroxo bridge increases the stability of the complex.[477] Results similar to those described for histidine are also obtained for 2,3-diaminopropionate as the ligand with which the dibridged species forms at pH >9.[493] Formation of a hydroxo bridge does not require a water-occupied coordination site for its occurrence but may also appear by driving a chelated carboxylate from the cobalt ion.

Solutions containing brown binuclear peroxo complexes of Co(III) eventually yield red mononuclear Co(III) complexes. The half-life for the reaction is about 16 min for histidylglycine, 6 hr for histidinamide, 7 hr for glycylhistidine, 9 hr for histidine, and 12 days for histamine.[473] With histidine hydrogen peroxide is not found in the final red solutions[494] and an additional mole of oxygen is required to produce the mononuclear complex.[495,496] Some ligand may be partially oxidized during the course of mononuclear Co(III) complex formation. Addition of H_2O_2 to solutions containing binuclear complexes speeds formation of mononuclear complexes.[493] It has been generally assumed that binuclear peroxo complexes are intermediates in the oxidation of Co(II) to mononuclear Co(III) complexes. Several lines of evidence suggest, however, that the binuclear peroxo complexes may not be the precursors of the mononuclear Co(III) complexes. The binuclear peroxo complexes may be relatively unreactive species produced in a side reaction not on the main reaction pathway for oxidation of Co(II) to red mononuclear Co(III) complexes.[493] Similarly, oxyhemoglobin may not be on the main pathway for oxidation of hemoglobin to methemoglobin.

The reduction potential of the aqueous Fe(III)/Fe(II) couple (+0.77 V) is reduced by ligands that are negatively charged or are stronger σ donors than water, such as amines. Because of its π-electron acceptor properties, imidazole, when compared to saturated amine donors, should favor the reduced state in Fe(III)/Fe(II) systems. The reduced state is more favored for the stronger π electron acceptor pyridine as exhibited by high positive reduction potentials for the tris(bipyridyl) and tris(o-phenanthroline) complexes. Tris complexes of the two ligands are low spin for both components of the Fe(III)/Fe(II) couple. The tendency of π-acceptor ligands to stabilize low-spin species is also displayed by imidazole when it appears as an axial ligand in iron porphyrin complexes. It is easier to effect the high-spin to low-spin conversion with Fe(II) than with Fe(III).

Near neutrality the reduction potentials of myoglobin and hemoglobin are about +0.1 V. Both proteins contain histidyl side chains in axial positions. Despite the significantly lower potential of the proteins they are oxidized by oxygen much more slowly than aqueous Fe(II). Thus the slower oxidation of the heme group in proteins is a kinetic effect. A model system with axial imidazole ligands attached to a heme group embedded in a hydrophobic matrix was found to combine reversibly with oxygen.[377] Kinetic features of the oxidation of heme systems have been discussed.[377] As for the reaction of Co(II) complexes with oxygen discussed above, it is possible that oxygenation of heme compounds represents a side reaction, not on the main pathway for oxidation of the metal ion. Complexation with added imidazole decreases the reduction potential of myoglobin due to the greater stability constant of imidazole with the Fe(III) protein.[419] Early studies of the effect of ligands on the redox potential of metalloporphyrins and hemoproteins have been summarized.[497]

The reduction potential of cytochrome c (+0.26 V) is more positive than that of the oxygen-carrying heme proteins. The detailed mechanism by which the reduced form undergoes rapid oxidation by good one-electron oxidants is uncertain. In section VII.C we mentioned the increase in oxidizability of carboxymethylated cytochrome c on complexation with imidazole. The modified protein itself is not rapidly oxidized by O_2, but after complexation with imidazole the protein is rapidly oxidized to the ferric state. These observations have led to the suggestion that the imidazole molecule may mediate the required electron transfer. This modified protein system then represents a case in which a major change in the reactivity of a porphyrin iron is caused by introduction of an imidazole ring as the axial ligand. However, since the identity of the axial ligands in the carboxymethylated protein is uncertain, as are conformational changes which may have occurred with carboxymethylation, it does not seem possible at this time to describe the structural basis of the reactivity change with assurance.

Imidazoles inhibit the catalase-like activity of cobalt(III) hematoporphyrin.[498] The inhibition of imidazole-containing compounds is believed to be qualitatively different from other types of inhibitors, such as pyridine. While the "normal" inhibitors are considered to act by competing with hydrogen peroxide for an axial coordination site at the metal ion, imidazole bases are oxidized by H_2O_2 when incorporated as an axial ligand. When this reaction occurs, oxygen is not evolved, but the catalytic activity of the complex is modified, and the net reaction is that of a peroxidase rather than a catalase.

An interesting, but as yet unexplained,[24] contrast in the behavior of imidazole ligands relative to pyridine derivatives has been noted in the reaction of salen complexes of Co(II) with alkyl halides. The normal mechanism for such reaction involves halogen atom transfer. When the

$$L_5Co^{II} + RX \longrightarrow L_5Co^{III}X + R\cdot$$
$$L_5Co^{II} + R\cdot \longrightarrow L_5Co^{III}\text{-}R \qquad (31)$$

halide is an o- or *p*-nitrobenzyl halide, and thus can act readily as a one-electron acceptor, a change of mechanism can occur.[499] The change in mechanism is ob-

$$Co^{II}(salen)(MeIm) + R'X \longrightarrow Co^{III}(salen)(MeIm) + [R'X]^{\cdot-}$$
$$R'X^{\cdot-} \longrightarrow R^{\cdot} + X^{-} \quad (32)$$
$$Co^{II}(salen)]MeIm) + R^{\cdot} \longrightarrow R\text{-}Co(salen)(MeIm)$$

served for Co(II) species with imidazole derivatives as axial ligands but not for the corresponding pyridine compounds, suggesting that the imidazole ring in some way facilitates the "outer-sphere" electron-transfer mechanism.

In summary, while there have been attempts to directly link imidazole rings to electron transfer mechanisms and although some studies suggest facets of uniqueness which might be the basis for a functional role of the imidazole ring, none of the model systems or biological systems have yet been described in such detail as to establish a special role for this ligand in biological redox processes.

IX. Metal Ion Induced Ionization of Pyrrole Hydrogen in Imidazole and Derivatives

More than 30 years ago, in order to account for a deprotonation in the imidazole complex of ferrihemoglobin occurring at pH 9.5,[500] it was suggested that ionization of a pyrrole nitrogen took place.[501] Other instances of the occurrence of this ionization in heme proteins have since been suggested, but only in the last 10 years have model compound and macromolecule studies elaborated the conditions and pK_a values at which metal ion induced pyrrole hydrogen ionizations occur in solution. Solid-state complexes with ionized pyrrole hydrogens are reviewed in sections III and IV. Many of the results obtained recently in solution are collected in Table XI. The pK_a values for the complexes are quoted for 25° and the ionic strength of the measurement from 0.04 to about 0.5 *M*. Technical difficulties make some of the constants less certain than customary for pK_a values.

Though much effort has been expended in attempting to evaluate pK_a values for pyrrole hydrogen ionization in simple complexes of imidazole and derivatives, values appear to be known for but a few cases. For many metal ions and most divalent ones of the first transition series, other reactions occur preferentially in the basic solutions required. Precipitation of hydroxides is inhibited for the three Co(II) complexes of small ligands in Table XI by the conversion from octahedral to tetrahedral geometry as pyrrole hydrogen ionization takes place in oxygen free solutions.[143] It is uncertain how many ligands on each complex give rise to a pyrrole hydrogen ionization, but the number two has been suggested so that the pK_a values of Table XI are an average of two ionizations. The octahedral to tetrahedral conversion is accompanied by a marked change and intensification of color from pale pink-yellow to deep violet to yield an absorption maximum near 570 nm in the visible spectrum.[143] Calculation indicates that these complexes in solution and those of imidazole[29,35] and benzimidazole[104] in the solid state yield the highest ligand field *Dq* parameter known in tetrahedral Co(II) systems. Ligands higher in the spectrochemical series do not appear to yield tetrahedral complexes with Co(II).

The acidity of the pyrrole hydrogen of imidazoles increases upon complexation of a metal ion at the pyridinium nitrogen. Co(II) lowers the pK_a from that of unbound ligand by about 2 log units, Cu(II) by 1 more log unit, and Pd(II) by almost another log unit. For all three metal ions comparison with complexes of related ligands such as 3-methylhistidine strongly suggests that the ionization ascribed to a pyrrole hydrogen in these complexes is identified correctly. Because the carboxylate side chains are unbound in the four-coordinate complexes, the formal charge at the divalent metal ion is 2+ for the first five complexes of Table XI. It seems evident that electronic effects related to covalency are more important than charge in determining the acidity of the pyrrole hydrogen. The still more strongly covalent Ru(III) complexes of higher formal charge yield the lowest pK_a values for the simple complexes.

TABLE XI. pK_a Values for Pyrrole Ionization in Imidazole and Derivatives

Compound	pK_a	Ref
Imidazole (Im)	14.2–14.5	13–15
Histidine (His)	14.4	13
$Co(Im)_4^{2+}$	12.5	143
$Co(histamine)_2^{2+}$	13	143
$Co(L\text{-}His)_2^{0}$	12.5	143
$Cu(L\text{-}His)_2^{0}$	11.7	143
$Pd(en)(L\text{-}His)^{+}$	10.8	156
$Ru(NH_3)_5Im^{3+}$	9.0	43
$Ru(NH_3)_5His^{2+}$	8.8	172
Cobalamin·Im	10.3	350
Cyanocobinamide·Im	11.5	347, 505
Cyanocobinamide·BzIm	9.5	347
Cyanocobinamide·histamine	11.1	505
Metmyoglobin·Im (sperm whale)	10.4	15
Methemoglobin·Im (*Chironomous plumosus*)	10.5	506

Pyrrole hydrogen ionizations have also been investigated in several other small complexes. In 50% ethanol the ionization that occurs with $pK_a = 14.2$ in 2-(2′-pyridyl)imidazole is reduced in 3:1 complexes to about $pK_a = 7.9$ for Cu(II) and $pK_a = 8.5$–9.0 for Fe(II), Co(II), Ni(II), and Zn(II).[502] In 50% dioxane the pyrrole ionization that occurs with $pK_a > 12$ in the free ligand 2-(2′-pyridyl)benzimidazole is lowered to $pK_a \sim 7$ in the Co(II), Ni(II), Cu(II), and Zn(II) complexes.[503] This low value is due to the electron-withdrawing effect of the benzene ring.

In all the complexes mentioned so far, pyrrole hydrogen ionization occurs across the imidazole ring from the metal ion binding site. Cases are also known where pyrrole hydrogen deprotonation occurs by substitution of the proton by a metal ion at the pyrrole nitrogen. In neutral solutions glycyl-L-histidine serves as a terdentate ligand with an amino, a deprotonated amide, and a pyridinium imidazole nitrogen donor atoms to yield tetragonal complexes of Ni(II), Cu(II), and Pd(II) of zero net charge.[504] All three 1:1 metal ion complexes undergo a concentration dependent deprotonation near pH 9.6. This deprotonation is accompanied by a significant shift to shorter wavelengths in the ligand field absorption spectra of the Cu(II) and Pd(II) complexes, indicative of replacement of water by a nitrogen donor atom in the fourth tetragonal coordination position. This transformation is best accommodated by substitution of the pyrrole hydrogen by the fourth coordination position about the metal ion to form a polymer, probably a tetramer.[504] These promoted pyrrole deprotonations due to substitution by a metal ion occurring at pH <10 are not included in Table XI because it is intended to refer to complexes where pyrrole ionization occurs freely without substitution by metal ion at the pyrrole nitrogen. For divalent metal ions of the last part of the first and second transition series deprotonation of a pyrrole hydrogen due to substitution by a metal ion occurs at pH <10 while ionizations induced by a metal ion across the imidazole ring take place at pH >10, as indicated in Table XI.

The second group of complexes in Table XI are related to vitamin B_{12} chemistry. In the aquocobalamin, which possesses a benzimidazole-Co(III) bond in the fifth coordination position, water in the sixth position is replaced to form the imidazole complex. Pyrrole hydrogen ionization from the complexed imidazole is promoted by 4 log units in this complex of 2+ formal charge at the metal ion. In the cyanocobinamide (factor B) complexes, the presence of CN^- reduces the formal charge at the metal ion to 1+. Pyrrole ionizations from the cyanocobinamide complexes where water in the sixth position is replaced by imidazole or histamine are promoted by about 3 log units. Because of the change in electronic character of the coordinating group in the fifth position, it seems uncertain to what extent the increase of about 1 log unit in promotion of pyrrole hydrogen ionization is to be attributed to the increase in formal charge by one unit in going from cyanocobinamide to cobalamin complexes. For three of the corrinoids, the increased pyrrole hydrogen acidity appears to be due mainly to a decrease in endothermicity.[350,505]

The last two entries of Table XI, a Fe(III)-myoglobin and a Fe(III)-hemoglobin, yield nearly identical pyrrole hydrogen ionizations for an added imidazole group coordinated in an axial position. The 4(5)-methylimidazole complex of the Fe(III)-hemoglobin undergoes pyrrole hydrogen ionization with $pK_a = 10.9$,[506] consistent with its greater basicity compared to imidazole (section III, Table II). Finally, imidazole complexed as an axial ligand to the diethyl ester of hemin in DMSO yields $pK_a = 10.4$.[507]

In conclusion we note that, except for the benzimidazole case, the pK_a values for the last five entries in Table XI, all macromolecules, exhibit values of $pK_a > 10$. These results permit us to expand the earlier deduction and conclude that pyrrole hydrogen ionizations induced by di- or trivalent metal ions of the first transition series and divalent metal ions of the second transition series complexed across the imidazole ring take place with $pK_a > 10$.

X. Addendum

From exchange of the H-2 proton of the imidazole ring with deuterium Ritsma[508] concludes that assignments of the contact shifted H-2 and H-4 protons of Co(II) complexed histidine in ref 33 should be reversed.

The tendencies toward mixed complex formation may be evaluated in terms of Δ log K values (Table VII) from information reported in several recent papers. For ternary complexes of Ni(II) and histidinate Δ log K values are as follows: aspartate, 0.57; glutamate, 0.87; methionine, 0.37; and tryptophan, 0.63.[509] Values for mixed complexes of Cu(II) and histidinate are serinate, 0.67, and glutamine, 0.34.[510] Ternary complexes of Cu(II) and histamine yield Δ log K values from 0.64 to 0.70 for the anions of glycine, alanine, α-aminobutyrate, and norvaline.[511] From thermochemical results presented in the last paper, the tendency toward mixed complex formation (CuAB) in the Δ log K formulation is associated with an unfavorable positive enthalpy change, ΔH = 1.8 to 2.1 kcal/mol, which is offset by a favorable positive entropy change, ΔS = 3 to 5 eu. For further discussion see ref 191.

Additional direct data indicating that imidazoles as axial bases facilitate O_2 binding by heme iron have been obtained. The oxygen binding ability of the myoglobin model shown in eq 24 was compared with a similar compound having a pyridine ring in place of the imidazole. The O_2 binding constant is at least 3800 times greater in the imidazole compound than in the pyridine analog. The two compounds have similar affinities for CO. The π-donor ability of the imidazole ring is suggested as an explanation.[512] A synthetic iron porphyrin with exceptional steric bulk on one side of the ring has been prepared. The 1-methylimidazole complex reversibly forms an oxygen complex.[513] The conclusions[379,385] regarding axial ligand effects on oxygen binding by porphinatocobalt(II) have been challenged,[514] but the original authors have reaffirmed their conclusions.[515]

An additional cobalt(II) system, *N,N'*-ethylenebis(benzoylacetiminato)cobalt(II), has been studied with respect to O_2 binding as a function of axial base. Two imidazoles were included, and both exhibited a greater effect in promoting oxygen binding by cobalt than would be expected if σ basicity were the governing factor.[516]

A crystal structure has been determined for 1-methylimidazole tetraphenylporphinatocobalt(II). The pentacoordinate cobalt is slightly displaced from the porphyrin coordination square toward the imidazole.[517]

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